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THE MECHANISM OF ELIMINATION OF CERTAIN STRAINS OR SPECIES OF TRYPANOSOMES WHEN MIXED IN EXPERIMENTAL INFECTIONS

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When mixed infections are established with trypanosomes differing in readily recognizable characters (such as presence or absence of a kinetoplast, sensitivity or resistance to drugs), one component of the mixture usually diminishes in relative numbers and disappears more or less rapidly (for review of some of the pertinent literature, see Cantrell, 1956). The mechanism responsible for this disappearance has apparently never been elucidated experimentally, although the view has been expressed repeatedly that differential growth rates may be the reason. An experimental analysis seemed desirable, since differential growth certainly is not the only mechanism which theoretically might explain the observed facts.

METHODS

The trypanosome mixtures required for the initial mixed infections were prepared by counting in a Levy counting chamber the trypanosomes in a blood sample from each of 2 stock rats infected with designated parasites and mixing appropriate amounts of the blood samples. A 50 : 50 ratio was used for most of our experiments. This inoculum then served to establish the initial infections which are called transfer O in the figures. From these animals serial transfers were made to new rats (or in 2 series, mice) twice weekly.

In most series the proportion between the 2 strains or species mixed was established at the time of each transfer. This was done by counting 500 specimens from Giemsa-stained blood smears when kinetoplast-containing *Trypanosoma equiperdum* USDA strain or *T. gambiense* Wellcome TS strain was mixed with kinetoplast-free *T. equiperdum* NIH strain (Tobie, 1951). This procedure could not be used when an arsenic-sensitive strain of *T. gambiense* Wellcome TS was mixed with the arsenic-resistant strain of the same species since the flagellates of both strains contained kinetoplasts. In this case the degree of respiratory inhibition produced by various concentrations of reduced tryparsamide (for procedure see von Brand et al, 1953) was established at the end of designated transfers and compared with the inhibition or lack thereof shown by the non-mixed strains. In some instances the in vitro resistance of the mixed and non-mixed flagellates to certain concentrations of the drug was also studied, as described earlier (Tobie and von Brand, 1953).

While infections with the parasites used produce fulminant infections in small rodents, they produce a more chronic type of infection in rabbits. The parasite density in rabbits is extremely low and direct differential counts of the *T. equiperdum* USDA and NIH strains inoculated as a 50 : 50 initial mixture in rabbits would have been impractical. Instead, a blood sample was withdrawn from an infected rabbit at designated times after infection and mice were injected with this blood. When the parasites had reached a fairly high density in the mice, blood smears were prepared and differential counts made as described above.

In order to examine the question whether or not a differential multiplication rate is respon-

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* With the technical assistance of Harvey D. James, Jr. and Kenneth E. Moats.

sible for the disappearance of one of the strains or species of a mixed infection, counts of the number of trypanosomes present in the blood of rats were made for each individual strain or species at 24 ± 0.5-hour intervals during the logarithmic period of development. At this time transfers of the mixed populations were made to fresh rats. The multiplication rate was then calculated according to the following formula, apparently used first by Buchner et al (1887): $n = \log N_t - \log N_0 / \log 2$; n is the number of generations, N_0 the number of parasites per unit volume at the time of the first count, and N_t the number of parasites per unit volume at time 0 + 24 hours. From the relation $N_t = N_0 \times 2^n$ (Buchner et al, 1887) the theoretical disappearance of the parasite with the lower multiplication rate was calculated according to the formula:

$$\%\ \text{strain 1 at transfer X+1} = \frac{\text{percent strain 1 at transfer X} \times 2^{n\ \text{strain 1}}}{(\%\ \text{strain 1 at transfer X} \times 2^{n\ \text{strain 1}}) + (\%\ \text{strain 2 at transfer X} \times 2^{n\ \text{strain 2}})}$$

n = number of generations between transfers X and X + 1.

RESULTS

1. *Multiplication Rate*

The multiplication rates of *T. equiperdum* USDA and NIH strains were established by counts on 12 rats and 12 mice each. There was no detectable difference in growth rate between parasites developing in rats and mice and the relevant values have therefore been averaged. The USDA strain divided 4.60 ± 0.11 times in 24 hours and the NIH strain 4.47 ± 0.18 times. The arsenic-sensitive strain of *T. gambiense,* based on 36 rats, divided 4.75 ± 0.10 times in 24 hours and the arsenic-resistant strain of the same species, based on 24 rats, 4.55 ± 0.11 times.

The differences between the strains are statistically not significant since the S value calculated according to the formula $S = M_1 - M_2 / \sqrt{SE_1^2 + SE_2^2}$ is smaller than 2.0 (0.6 + 1.3, respectively). They may nevertheless be real, within reason-

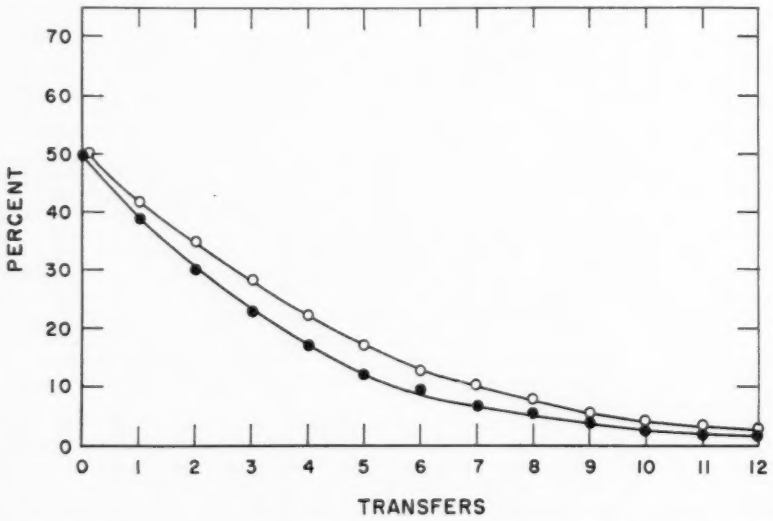

FIGURE 1. Disappearance of *T. equiperdum* NIH strain from an initial 50:50 mixture of *T. equiperdum* NIH strain and *T. equiperdum* USDA strain.

° Calculated from observed multiplication rates (*T. equiperdum* NIH strain: 4.47; *T. equiperdum* USDA strain: 4.60).

• Observed rate of disappearance.

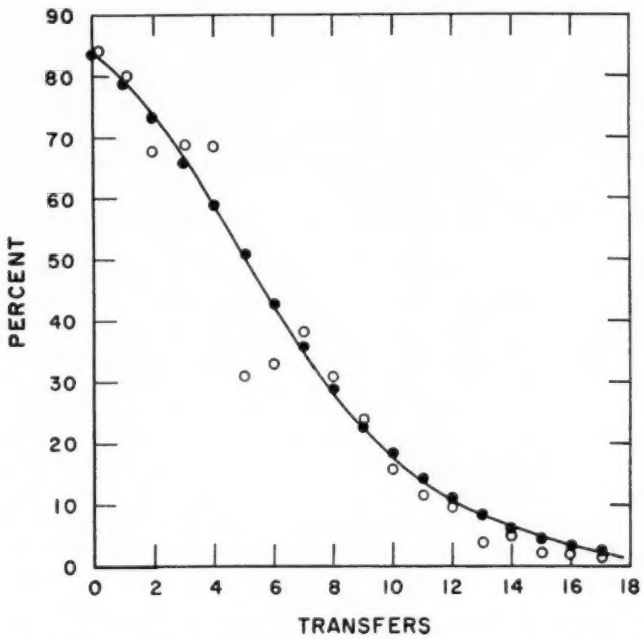

FIGURE 2. Disappearance of *T. equiperdum* NIH strain from an initial 84: 16 mixture of *T. equiperdum* NIH strain and *T. equiperdum* USDA strain.
• Calculated from observed multiplication rates (*T. equiperdum* NIH strain: 4.47; *T. equiperdum* USDA strain: 4.60).
° Observed rate of disappearance.

able limits of error. All observations detailed below can be explained on the assumption that loss of the kinetoplast or induction of arsenic-resistance is responsible for a slightly lower multiplication rate than found in the parent strains.

2. *Mixtures of* T. equiperdum *USDA and NIH Strains*

Three series with an initial 50: 50 mixture of both strains were done, 2 utilizing rats and 1 mice. All gave analogous results and their values have therefore been averaged. As figure 1 shows, the percentage of the kinetoplast-free parasites in the population progressively decreased and there was close agreement between the

TABLE I. *Percentage of kinetoplast-free* Trypanosoma equiperdum *in the blood of mice injected with blood from rabbits which had been infected with a 50: 50 mixture of kinetoplast-containing and kinetoplast-free strains.*

Time in weeks after inoculation of rabbit	Kinetoplast-free trypanosomes as percentage of trypanosome population in mice injected with blood from rabbit number				
	1	2	3	4	5
1	1.0	2.0	2.2	3.1	4.0
2	2.4	7.0	2.0	2.8	0.4
3	5.2	5.6	5.8	20.2	0.4
4	2.4	1.8	Dead	2.4	0.4
5	2.8	1.4		2.8	3.0
6	Dead	0.6		1.2	1.6
7		Dead		1.2	0.6
8				Dead	Dead

observed rate of reduction and the theoretical one, calculated from the division rates of both strains.

In 1 mouse series (fig. 2) in which the strains were mixed in the proportion of 16 USDA to 84 NIH initially, the first value and all values between 7th and 17th transfers followed closely the calculated curve, but the values of the 4th, 5th, and 6th transfers deviated markedly from it. No definite explanation can be offered, but it should be kept in mind that the values are derived from one series only; any experimental error and any biological variation would therefore show up in full measure.

A final series with a 50:50 mixture of both strains was done in rabbits, and the results were evaluated from weekly inoculations of rabbit blood into mice. As table I shows, the results were quite variable, especially during the first 3 weeks when the proportion of kinetoplast-free trypanosomes varied between 0.4 and 20.2 percent of the population. From the scarcity of the higher values for this strain, it is apparent that it diminished rather rapidly, just as it did in rats. The occurrence of occasional high values does not indicate to us that at times the NIH strain divided more rapidly in rabbits than the USDA strain. We are inclined to explain the occurrence of these variations on an entirely different basis. As mentioned, trypanosomes are extremely rare in the peripheral blood of rabbits; in fact, it is often very difficult to find them microscopically. Since a limited amount of blood can be injected into a mouse, only a few trypanosomes will be transferred from rabbit

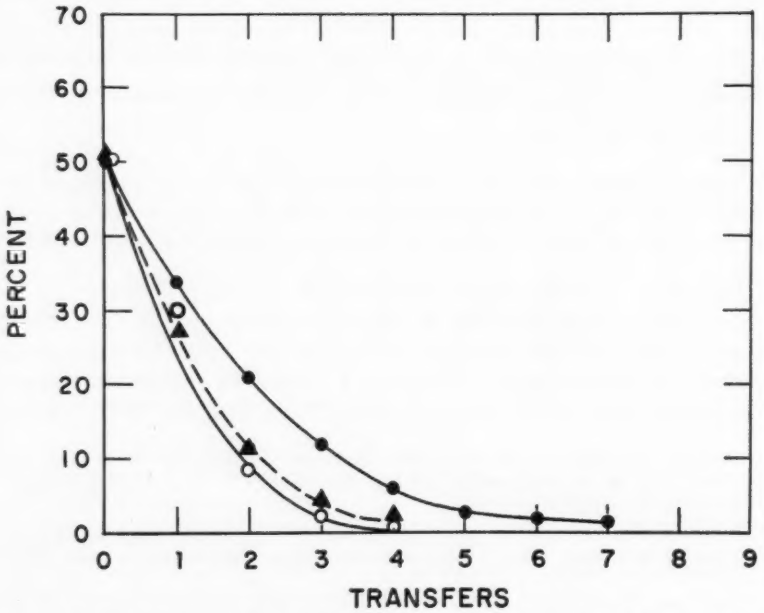

FIGURE 3. Disappearance of *T. equiperdum* NIH strain from an initial 50:50 mixture of *T. gambiense* arsenic-sensitive strain and *T. equiperdum* NIH strain.
● Calculated from observed multiplication rates (*T. gambiense* arsenic-sensitive strain: 4.75; *T. equiperdum* NIH strain: 4.47).
▲ Calculated from assumed multiplication rate for *T. gambiense*, arsenic-sensitive strain: (4.85) and observed multiplication rate of *T. equiperdum* NIH strain: (4.47).
○ Observed rate of disappearance.

to mouse and the chance distribution of the 2 strains in the blood inoculated will decide largely the percentage of each, as shown in a few days by the recipient mouse. It is evident that the material does not lend itself to further analysis. Specifically, it is impossible to tell whether or not the influence emanating from the rabbit which keeps the trypanosome population low (destruction of trypanosomes, induction of slower division, or some other mechanism) acts differentially on both strains.

3. *Mixture of* T. equiperdum *NIH Strain and* T. gambiense *Wellcome TS Strain*

The 2 relevant series with a 50: 50 mixture of *T. equiperdum* and *T. gambiense* were done in rats. The results of both series coincided very closely and are therefore presented as averages in figure 3. It is evident that *T. equiperdum* was very rapidly reduced to such an extent that after 4 transfers it had practically disappeared. The rate of disapearance was somewhat faster than expected from the multiplication rates. This may be due to a small error in the multiplication figure of *T. gambiense*. If the daily division rate of *T. gambiense* is assumed to be 4.85 instead of 4.75, that is if it is increased by a figure corresponding to one times the standard error of the mean, a surprisingly close agreement to the observed rate of disappearance exists (fig. 3).

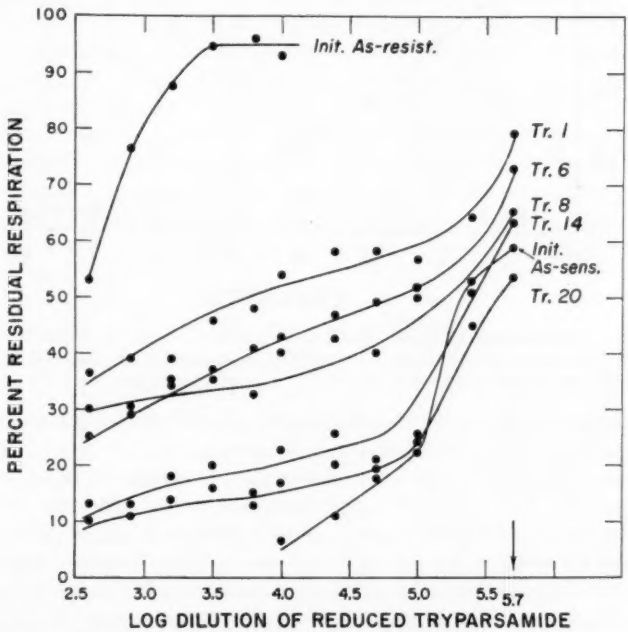

FIGURE 4. Respiration, in percent of non-inhibited rate, under the influence of specified concentrations of reduced tryparsamide, of arsenic-resistant, arsenic-sensitive *T. gambiense*, and of mixed populations at designated transfers (initial mixture 50: 50 arsenic-resistant and sensitive).

4. *Mixture of* T. gambiense *Wellcome TS, Arsenic-Sensitive, and Arsenic-Resistant Strains*

Three series were done in rats with 50: 50 mixtures of the above 2 strains. At

designated transfers the sensitivity of the mixed strain's respiration to a series of concentrations of reduced tryparsamide was tested. At alternate exposures, its sensitivity when exposed in vitro to 4 concentrations of the drug was studied. The results of the 3 series have been averaged and the respiratory studies have been summarized in figure 4. It is evident that the sensitivity of the mixed strain increased with time, indicating a gradual diminution of the arsenic-resistant strain in the population. This is in accord with the behavior deduced theoretically from the multiplication rates of both strains (fig. 5). The material is not suitable for a

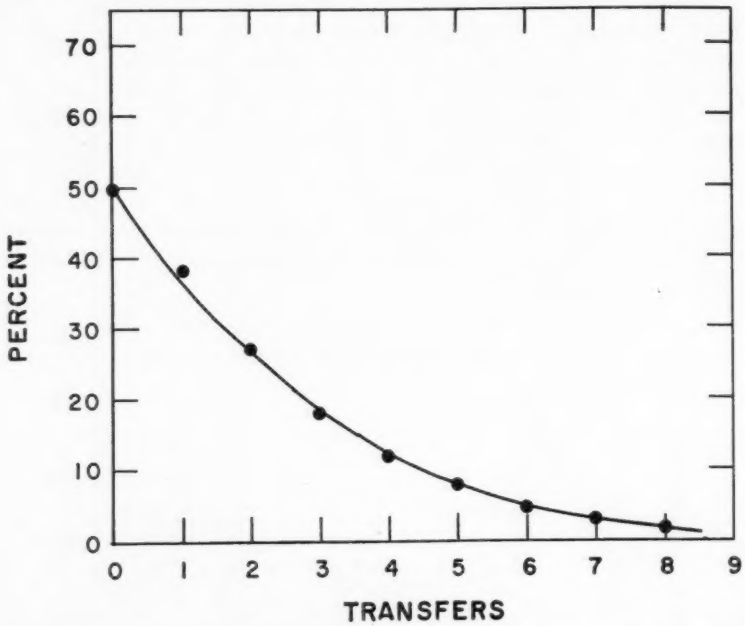

FIGURE 5. Disappearance of arsenic-resistant *T. gambiense* from an initial 50:50 mixture of arsenic-resistant and arsenic-sensitive *T. gambiense*. Calculated from observed multiplication rates (*T. gambiense* arsenic-resistant strain: 4.55; *T. gambiense* arsenic-sensitive strain. 4.75).

detailed analysis concerning the degree of agreement or disagreement between observed and theoretical reduction of the arsenic-resistant strain. It does seem, however, that the reduction is somewhat slower than expected. The results of the in vitro exposures (averages of 2 series) are presented in table II. A very slow trend towards decreased numbers surviving the exposures is clearly present. Many trypanosomes surviving in the highest drug concentration, however, had lost their power to infect experimental animals. On the other hand, as shown by the results of transfer 30, occasionally trypanosomes capable of developing in experimental animals were present in too small numbers for counting, but microscopical scrutiny of a drop of medium studied at the end of the in vitro exposure showed some motile flagellates. A strain multiplying slightly more slowly than another one will rapidly diminish in numbers to such an extent that it may become undetectable by such relatively crude yardsticks as counting kinetoplast-containing and kinetoplast-free specimens or determining respiratory inhibitions produced by a few

TABLE II. *Resistance to reduced tryparsamide in vitro of* Trypanosoma gambiense *Wellcome TS arsenic-sensitive, arsenic-resistant strains, and of the mixed populatoin begun as a 50:50 mixture at designated transfers.* (Figures are percentage of motile flagellates as compared to control not exposed to drug; + indicates that rats were infected by survivors; − that rats were not infected.)

Strain	Exposure at time of transfer number	Percent and infectivity of survivors exposed to specified dilutions of reduced tryparsamide			
		1 : 3,125	1 : 12,500	1 : 50,000	1 : 200,000
Arsenic-sensitive	—	0 −	0 −	0 −	0 −
Arsenic-resistant	—	77 +	103 +	100 +	130 +
Mixed	3	30 +	48 +	30 +	35 +
"	5	40 −	32 +	32 +	40 +
"	7	12 −	6 +	23 +	26 +
"	9	24 −	22 +	22 +	27 +
"	11	17 −	14 +	27 +	21 +
"	13	24 −	8 +	10 +	12 +
"	15	4 −	7 +	16 +	11 +
"	17	11 −	9 +	15 +	11 +
"	19	3 −	6 +	3 +	3 +
"	21	5 −	6 +	14 +	10 +
"	30	0 +	0 +	3 +	5 +
"	38	0 −	0 −	0 −	0 −

drug concentrations. However, the diminishing strain will still be a minor component of the mixture for a long time, depending on how many trypanosomes are used to make the transfers. In the present case it is characteristic that up to the time of total disappearance, as judged by the methods used, infective trypanosomes occurred with regularity in the 3 drug dilutions which had not killed any of the arsenic-resistant members of the parent strain. The highest drug concentration was damaging even to the trypanosomes of the parent strain, more so actually than indicated by the condensed presentation of our data in table II. Actually only 1 of 2 rats became infected after inoculation with the surviving trypanosomes, and it survived exceptionally long showing that many of the surviving flagellates had been damaged by the exposure and were not infective. With the smaller numbers of resistant trypanosomes expected in transfers of the mixed strain, it does not seem surprising that in many transfers no viable specimens were left after exposure to the highest drug concentration.

DISCUSSION AND SUMMARY

Our experiments have shown that when an unaltered strain of trypanosomes is mixed with a strain that either has lost its kinetoplast or that has been rendered arsenic-resistant, the strain with changed characteristics invariably, and more or less rapidly, disappears from a population maintained by serial transfers in rats or mice. This is in accordance with the findings of most authors (literature in Cantrell, 1956), although a few opposite findings have also been reported (Laveran and Roudsky, 1912; Teichmann, 1918). Tseng (1935) investigated mixtures of various species of trypanosomes. In mixtures of *T. gambiense, T. lewisi,* and *T. cruzi* no clear cut displacement was observed; in mixtures of *T. brucei* and *T. congolense,* however, the latter always disappeared rapidly.

Theoretically, at least 3 possibilities for the loss of a strain from a mixture exist: differential reproductive rates (Oehler, 1914), obliteration of a long-lasting modification (Jollos, 1921) in consequence of a sexual process, or disappearance of a genetically fixed recessive character due to hybridization. The occurrence of sexual processes in trypanosomes has been claimed several times (Culwick et al,

1951; Fairbairn et al, 1946; Fiennes, 1945), but is not generally accepted (Fromentin, 1955; Amrein, 1957). Our experiments have shown that in those instances where a closer analysis was possible, the observed rate of disappearance closely followed the rate of disappearance calculated from our determinations of the multiplication rates of the parent strain. Those cases that for various reasons could not be analyzed in reference to the multiplication rate of the parent strains do not contradict the assumption that differential multiplication lies at the root of the observed phenomena. None of the observed facts requires the assumption of intervening sexual processes. The mechanism heretofore assumed as responsible on theoretical grounds (Cantrell, 1956), namely slight differences in multiplication, has now been given experimental support.

References

Amrein, Y. U. 1957 Evidence against sexuality in *Trypanosoma gambiense*. J. Prot. **4:** 67–68.

Buchner, H., Longard, K. and Riedlin, G. 1887 Ueber die Vermehrungsgeschwindigkeit der Bacterien. Cbl. Bakt. **2:** 1–7.

von Brand, T., Tobie, E. J., Mehlman, B. and Weinbach, E. C. 1953 Observations on the metabolism of normal and arsenic-resistant *Trypanosoma gambiense*. J. Cell. and Comp. Physiol. **41:** 1–22.

Cantrell, W. 1956 Behavior of mixtures of oxophenarsine-resistant and unmodified strains of *Trypanosoma equiperdum* in the rat. Exp. Parasit. **5:** 178–190.

Culwick, A. T., Fairbairn, A. and Culwick, R. E. 1951 The genetic relationship of the polymorphic trypanosomes and its practical implications. Ann. Trop. Med. Parasit. **45:** 11–29.

Fairbairn, H. and Culwick, A. T. 1946 A new approach to trypanosomiasis. With a statistical analysis by F. L. Gee. Ann. Trop. Med. Parasit. **40:** 421–452.

Fiennes, R. N. T.-W. 1945 A sexual reproduction cycle of *T. congolense*. Nature **156:** 390.

Fromentin, H. 1955 Essais de création d'une souche "hybride" par mélange de trypanosomes polymorphes d'espèces différentes. Bull. Soc. Path. Exot. **48:** 414–421.

Jollos, V. 1921 Experimentelle Protistenstudien. 1. Untersuchungen über Variablilität und Vererbung bei Infusorien. Arch. Prot. **43:** 1–222.

Laveran, A. and Roudsky, D. 1912 Résultats obtenus en mélangeant un virus à trypanosomes acentrosomiques avec un virus normal de même espéce. Compt. Rend. Soc. Biol. Paris **72:** 313–314.

Oehler, R. 1914 Untersuchungen über den Dimorphismus von *Trypanosoma brucei*. Ztschr. Hyg. u. Infkr. **77:** 356–370.

Teichman, E. 1918 Mischinfektionsversuche mit Trypanosomen. Ztschr. Hyg. u. Infkr. **82:** 511–526.

Tobie, E. J. 1951 Loss of the kinetoplast in a strain of *Trypanosoma equiperdum*. Tr. Am. Micr. Soc. **70:** 251–254.

——— and von Brand, T. 1953 The development of arsenic resistance in *Trypanosoma gambiense* and its influence on parasitemia. J. Inf. Dis. **92:** 132–138.

Tseng, H. 1935 Ueber die gegenseitige Beeinflussung verschiedener Trypanosomen bei Mischinfektion. Zbl. Bakt. 1. Abt. Orig. **134:** 153–159.

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: FURTHER OBSERVATIONS ON THE UPTAKE OF GLUCOSE BY *HYMENOLEPIS DIMINUTA**

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As has been pointed out in a series of papers by Read and his associates, carbohydrate is a necessary constituent of the host's diet for adequate growth and reproduction of tapeworms (Read, Schiller and Phifer, 1958). Since the tapeworm possesses no alimentary canal, this animal necessarily must depend upon the penetration of nutrient materials across its membranes. In an earlier study (Phifer, 1959), it was shown that, in the case of *Hymenolepis diminuta,* physical diffusion does not account for the observed uptake of glucose.

Although the data suggest that the process of glucose uptake in *H. diminuta* may be an "active" one, more information is necessary in order to substantiate this hypothesis. For this reason, experiments were carried out in which the study of the effects of chemical factors was extended to include the effects of inhibitors on the process of glucose absorption. Since the most important means of showing that a permeation process is an active one is the demonstration that the tissue accumulates the substance against a concentration gradient, experiments were carried out to determine whether such concentration occurred. In addition, the study of physiological factors was extended to determine whether uptake activity is affected by the location along the surface of the tapeworm.

MATERIALS AND METHODS

The cysticercoids of the tapeworm, *Hymenolepis diminuta,* were reared in the beetle *Tribolium confusum.* Each rat host was infected with 10 cysticercoids. The worms were then allowed to grow to the definitive "adult" size (ca. 20 days).

The two general experimental methods utilized were similar to those described by Phifer (1959). Incubations of worms were carried out for periods of 30 minutes and chemical analyses (Nelson, 1944) performed to determine the disappearance of glucose from the suspending medium; such experiments are referred to as "extended time" experiments. The second method involved the use of uniformly labelled C^{14}-glucose. The worms were incubated for 60 seconds and the amount of labelled compound taken up by the worms was extracted at the termination of the incubation; such experiments are referred to as "short-interval" experiments.

In order to determine more exactly that the substance being measured in each type of experiment was actually glucose, chromatographic and radioautographic analyses were carried out. Using the method of Bacon and Edelman (1951), samples of Nelson-positive material were spotted on Whatman No. 1 filter paper together with known glucose samples. The papers were placed in solvent (n-propanol, 7 parts; ethyl acetate, 1 part; water, 2 parts), developed for 13 hours, dried, and then sprayed with a solution composed of 0.5 g of benzidine in 10 ml of acetic acid, 10 ml of 40 percent aqueous trichloracetic acid, and 80 ml of ethanol. After drying,

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the developed spots showed that the samples were actually glucose and not another reducing substance which might yield a false positive reaction with the Nelson procedure.

In the case of the short interval experiments, radioautographic analysis was necessary. Extracted radioactive material and known samples of uniformly labelled glucose were concentrated and were spotted for chromatography as described above. After chromatographing, the paper was dried and a radioautograph of the chromatogram prepared by exposing Eastman No-screen X-ray film to the chromatogram for 2 weeks. It was seen that radioactivity of the experimental samples migrated as one component in the same location as the known glucose samples. In addition, each spot was carefully eluted in 50 percent alcohol, plated, and the amount of radioactivity was measured. These counts were then compared with the original values obtained from the extract before concentration, radioautography, and re-extraction of the spot on the chromatogram. It was seen that after 60 seconds the amount of radioactive material taken up by the worm was still 95 percent glucose. In other words, the use of this time interval virtually eliminated the catabolism of glucose as a complicating factor in the observed uptake phenomena.

RESULTS

On the basis of the early work of Lundsgaard on the severe effect of iodoacetate on carbohydrate metabolism, Wilbrandt and Laszt (1933) studied the effect of iodoacetate on absorption of glucose in the gut and found that absorption is greatly impaired by this compound. Walker and Hudson (1937) showed that a solution containing 1:5000 iodoacetate brought about diminished ability of frog kidney to reabsorb glucose.

Extended time experiments were carried out with *Hymenolepis diminuta* in which worms were incubated in 0, 10^{-3}M, and 3×10^{-4}M iodoacetate in order to see whether this metabolic poison would reduce the rate of glucose disappearance. Table I shows that iodoacetate greatly lowers the rate of glucose disappearance from

TABLE I. *The effect of iodacetate on the glucose uptake of* H. diminuta.

A. EXTENDED TIME EXPERIMENTS Uptake in micrograms of glucose per mg/hr.		B. SHORT INTERVAL EXPERIMENTS Uptake in CPM/mg per minute.	
UPTAKE RATE (Mean, S.D.)		UPTAKE RATE (Mean, S.D.)	
Glucose 8.3×10^{-3}M	116.0 (7.2)	Glucose 10^{-3}M	128.8 (8.2)
Glucose and 10^{-3}M iodoacetate	5.0 (3.6)	Glucose 10^{-3}M iodoacetate 10^{-4}M	119.3 (20.6)
Glucose and 3×10^{-4}M iodoacetate	33.6 (7.5)	Glucose 10^{-3}M iodoacetate 10^{-4}M worms were preincubated in 10^{-4}M iodoacetate for 15 minutes prior to the experiments	60.2 (14.1)

the medium. The data in table II compare the effects of iodoacetate on the glucose

TABLE II. *Uptake of glucose and the effect of a metabolic inhibitor on normal and heated tapeworms.*

	Normal worms	Worms heated at 60° C for 5 minutes
Glucose 8×10^{-3}M	73.3	16.2
	111.7	7.7
	138.2	13.2
	81.4	9.1
Glucose 8×10^{-3}M and Iodoacetate 3×10^{-4}M	47.4	8.5
	36.3	10.5
	29.1	5.1
	22.8	9.0

Each worm was incubated for 30 minutes at 37° C. and pH 7.5. The uptake values are expressed as micrograms of glucose per milligram of dry weight of worm tissue per hour.

uptake by normal and heat-killed worms.

In order to see whether iodoacetate would inhibit as in the extended time experiments, short interval experiments were undertaken in which 1 group of worms

was incubated for 60 seconds in 10^{-3}M glucose, and 2 groups in 10^{-3}M glucose and 10^{-4}M iodoacetate. One of the experimental groups was preincubated in 10^{-4}M iodoacetate for 15 minutes prior to the actual incubation. The data obtained are shown in table I. It may be seen that unless the inhibitor is added before the substrate, an insignificant inhibition of glucose uptake occurs.

Short interval experiments on the effect of dinitrophenol (DNP) were carried out in the same manner as used in testing the effect of iodoacetate. The worms were divided into 3 groups, (1) control, (2) preincubation in DNP and incubation in DNP, and (3) incubation in DNP without preincubation. It was found that, under the conditions of this experiment, DNP does not inhibit the uptake of glucose by *H. diminuta,* although DNP inhibition did occur in extended time experiments (Phifer, 1959).

One of the theories as to the mechanism of glucose absorption is that of an intermediary phosphorylation-dephosphorylation directly involved in the process (Höber, 1945; Wilbrandt, 1954; Rothstein and Meier, 1949). In a study of the possible mechanisms of glucose uptake by *H. diminuta* (Phifer, 1960), it has been shown that 6.5×10^{-4}M ammonium molybdate inhibits the cell-surface phosphatase of *H. diminuta.* Therefore, if phosphatases are involved in glucose absorption, the process should be inhibited by concentrations of molybdate which inhibit phosphatase. Hence, an experiment was set up in which 6.5×10^{-4}M ammonium molybdate was added to the glucose incubation media. The data showed that at this concentration molybdate does not affect glucose uptake by *Hymenolepis diminuta.*

It has been reported (Rothman, 1958) that bile salts inhibit the anaerobic glycolysis of exogenous glucose in *H. diminuta.* He reported that this inhibition could be reversed by a brief washing; these results suggest the possibility that bile salts act at the surface of the worm in inhibiting glycolysis. An experiment was designed to test this hypothesis. One group of worms was preincubated for 15 minutes in sodium taurocholate, and then each worm was incubated for 60 seconds in C^{14} labelled glucose and sodium taurocholate. Worms from a second group were treated in the same manner with the exception that they were not preincubated in taurocholate prior to incubation. The worms of the third group were each incubated in glucose alone. The data shown in table III indicate that this bile salt may not act at the surface of the worm since only in the preincubation group was there significant inhibition.

Since the tapeworm is physiologically most active at the region of the neck and scolex by virtue of the fact that this is the location of production of new segments, it might be supposed that the absorption of glucose, a utilizable energy source, would be greatest in the anterior region of the worm. In order to test this idea, 2 worms were cut into 4 equal sections and each was incubated in labelled glucose for 60 seconds. The data from this experiment, shown in table IV, support the hypothesis that the anterior portion of the worm takes up glucose at a much greater rate than do the other segments. In order to eliminate the possibility of introducing experimental error by measuring the uptake rates of cut pieces of worms, another experiment was designed. These tapeworms were each incubated in labelled glucose for 60 seconds. At the termination of incubation, each worm was cut into 4 equal parts and put into 4 tubes containing extraction fluid. The results of this experiment (table IV) corroborate very well those of the preceding experiment. Although previous experiments had shown that a small tapeworm does not absorb glucose at

TABLE III. *The effect of sodium taurocholate on the amount of glucose taken up by* Hymenolepis diminuta *in 60 seconds.*

Experimental variable	Uptake of C^{14} glucose (CPM/mg)	Mean value
Glucose 2×10^{-3}M	96.7 98.8 61.9 61.9 170.2 160.2	106.6
Glucose 2×10^{-3}M Sodium taurocholate 0.5 pct.	37.1 32.1 25.7	29.9
Preincubation in taurocholate for 15 minutes prior to experiment	27.2 30.7 26.8	
Glucose 2×10^{-3}M and Sodium taurocholate 0.5 pct.	100.3 91.4 104.4 101.3 66.6 67.3	88.6

This experiment was carried out at pH 7.0 and 37° C.

TABLE IV. *Glucose uptake by different parts of the strobila of* H. diminuta.

Experiment	Anterior fourth	Second fourth	Third fourth	Posterior fourth
Worms cut up before incubation	148.6 325.4	112.3 163.9	102.3 147.4	80.6 103.8
Worms cut up after incubation	149.9 215.6 188.0	103.3 120.2 115.5	93.1 96.4 87.8	71.1 78.2 64.0
	Worm weight		Uptake	
	Anterior	Posterior	Anterior	Posterior
Worms cut up after incubation	6.2 5.3 7.9 10.1 10.9 5.8	9.9 6.8 8.0 14.1 8.3 4.5	121.1 220.4 203.7 150.3 140.0 278.6	43.0 51.9 70.1 36.8 51.7 79.0

The glucose uptake is expressed in CPM/mg taken up by the tapeworm in 60 seconds.

a higher rate than a larger worm (Phifer, 1959), the weights of the tapeworms used were not so small as those in these gradient experiments. Therefore, an experiment was designed so that 2 pieces were cut from each of 6 worms after each worm had been incubated for 60 seconds in labelled glucose. The 2 pieces were cut from each worm so that they would be almost equal in weight, and so that one piece would be from the most anterior section of the worm and the other from the most posterior portion. The data, seen in table IV, show that the anterior portion takes up glucose at a rate over 3 times that of the posterior portion.

Rosenberg (1954) has pointed out that in an analysis of a transport process the most important means of showing that the process is an active one is demonstrating that the tissue accumulates the substance against a concentration gradient. This would imply that the addition of energy would be necessary for the uptake to occur. Attempts were made to determine whether glucose uptake is reversible. Worms were incubated in labelled glucose for 120 seconds, extracted, and then counted. In presenting the data in table V, the assumption is made that the density of a tape-

TABLE V. *Apparent accumulation of C^{14} Glucose by* Hymenolepis diminuta *at 37° C, pH 7.5, over a two-minute period.*

	CPM/ml	Mean value	Corrected for metabolism	Micrograms of glucose/ml
Medium	2946	2748		18
"	2570			
Worm No. 1	3948			
2	3885			
3	3780			
4	3843	4216	3689	24
5	4116			
6	3948	S.E. = 197.3	S.E. = 194.6	
7	5040			
8	5167			

The assumption is made that the density of the worm is 1.0, i.e., CPM/gm = CPM/ml.

worm is 1.0; but since it is obvious that the density of a tapeworm would be greater than 1.0, these data are minimal values. The figures are expressed as CPM/gm of wet weight which is equal to CPM/ml of worm tissue in order to compare the values from the worm with values of samples taken from the medium. It can be seen that the concentration inside the worm is apparently greater than that outside the worm.

In support of the above finding, an experiment was carried out in which 12 worms were each incubated for 60 seconds in labelled glucose. At the end of this time, 6 worms were rinsed, blotted, and placed in fresh Krebs Ringer solution without sugar for 60 seconds, and 6 were placed in tubes for extraction directly after incubation in the C^{14} glucose. In other words, these former 6 worms were placed in a medium in which the concentration gradient favored the flow of glucose from the worm. However, as can be seen from table VI, no significant passage of glu-

TABLE VI. *The effect of incubation in glucose-free media on the retention of glucose by the tapeworm,* Hymenolepis diminuta.

Controls	Worms placed in glucose-free Krebs-Ringer for 60 seconds
1—78.3 CPM/mg	7—72.6 CPM/mg
2—78.2	8—83.6
3—67.3	9—82.9
4—89.7	10—59.0
5—75.3	11—59.8
6—97.2	12—60.2

Each worm was incubated in a 2×10^{-3}M glucose C^{14} solution for 60 seconds at 37° C. and pH 7.5.

cose out of the worm occurred. These two experiments furnish evidence that *Hymenolepis diminuta* may transport and accumulate glucose against a concentration gradient.

DISCUSSION

It has been demonstrated that in the case of *Hymenolepis diminuta* the movement of glucose from the medium into the worm appears to be related to metabolic processes rather than to purely physical processes (Phifer, 1959). The studies on the effects of chemical factors on uptake further support this hypothesis. Iodoacetate, a potent inhibitor of sulfhydryl enzymes, was shown to inhibit glucose uptake by this worm, although in short term incubations a preincubation period was necessary in order to demonstrate inhibition. This might be an indication that iodoacetate does not act on a surface mechanism, but rather affects the general metabolic activity of the tapeworm. The data in table II show that in worms which had been heated, uptake rates were not affected by iodoacetate. Thus, it appears that metab-

olism is in some way related to glucose uptake.

Although DNP had been shown to reduce the level of glucose uptake in extended time experiments (Phifer, 1959), the data indicated that DNP does not act at the surface and that a preincubation period of 15 minutes is not of sufficient length to bring about a reduction of uptake activity. This might possibly be explained by postulating that the supply of endogenous high energy phosphate bonds is not depleted in 15 minutes; but after 30 minutes suppression of oxidative phosphorylation, and thus of production of new energy rich compounds, would begin to affect glucose uptake.

The fact that ammonium molybdate had no influence on glucose uptake by this tapeworm would tend to weaken the hypothesis that phosphatase is directly involved in glucose uptake, since at this concentration ammonium molybdate greatly reduces the surface phosphatase activity in *H. diminuta* (Phifer, 1960).

Rothman (1958) reported that bile salts inhibit the anaerobic glycolysis of *H. diminuta,* and that this inhibition could be reversed by a brief washing. From these data alone, one might conclude that this action of bile salts occurs at the surface of tapeworms. The data presented in table III indicate that this may not be the case. It was shown that the inhibition of glucose uptake by 0.5 percent sodium taurocholate was 4 times greater when the worms had been preincubated for 15 minutes prior to the 60-second incubation than when this preincubation had been omitted. This would suggest that the action of this bile salt is not on a surface mechanism, but on processes inside the worm, possibly involving denaturation of proteins (enzymes?).

The data of Gustavson et al (1957) tend to support the concept that there is a progressive decrease in bile salt concentration along the small intestine. Rothman (1958) presented the hypothesis that this decrease in bile salt concentration might be involved in the localization of *H. diminuta* in the lower part of the small intestine. He also noted the correlation between higher metabolic rates in worms which have been starved and decreased bile flow during starvation (Smith et al, 1928). Starvation of the host also results in increased glucose uptake rates in the worms. Although the main cause is probably depletion of endogenous polysaccharide, bile salt may also be important as a factor. It would be of interest to compare the effects of bile salts on the glucose uptake of *Hymenolepis citelli,* since it was found that bile salts do not affect the glycolysis of this species (Rothman, 1958).

The demonstration that anterior portions of *H. diminuta* take up glucose at greater rates than do posterior segments of this worm might be related to the hypothesis that the greatest synthesis is occurring in the neck region of the worm where production of new proglottids occurs. Another possible advantage to the worm of having a relatively greater amount of glucose taken up at the anterior end is that of furnishing an abundance of energy source to the scolex which presumably expends energy in the process of attachment to the host.

Since it has been shown that over a considerable range, concentration of glucose does not affect glucose uptake as would be the case in diffusion; heating the worms reduces uptake rates; temperature coefficients of uptake activity resemble those of chemical reactions; metabolic inhibitors reduce uptake rates and physiological factors affect uptake rates, it seems reasonable to suggest that glucose uptake in *H. diminuta* is coupled to energy metabolism. However, the only certain criterion of an active transport process is the demonstration of transport from a lower to a

higher chemical potential since this would necessarily involve the participation of forces other than diffusion (Rosenberg, 1954). Clark (1922) showed glomerular permeability to glucose even when the capillaries contained glucose at 9 to 10 times that of the normal blood level. Recently, Krane and Crane (1959) have shown that mammalian kidney slices accumulate galactose against a concentration gradient.

Although the data presented in table V show a significant apparent accumulation against a gradient, it is obvious from the variation that more experiments of this type are necessary before it can be concluded with certainty that active transport is responsible for the uptake of glucose by *H. diminuta.* Supporting data presented in table VI, show that this accumulated glucose is maintained within the tapeworm, when the concentration gradient favors the passage of glucose from the worm to the medium. It is of course possible that, once the glucose is transported across the membrane, it is trapped by binding, but still could be extractable in 50 percent ethanol.

If *H. diminuta* concentrates glucose against a gradient, it is in an advantageous position with respect to survival. If the concentration of a substance in the intestine were to fall to a very low level, such a process of membrane transport might permit the parasite to survive, whereas survival would be difficult or impossible if diffusion were the only means of transport. The size of the glucose molecule limits its diffusibility in the first place and, since diffusion is directly proportional to concentration, glucose could be expected to diffuse very slowly indeed at a low concentration. On the other hand, a tapeworm equipped with a catalytic mechanism of transport in an intestine containing a low concentration of glucose might obtain a sufficient quantity of the nutrient to remain established in the gut. Also, if the tapeworm had to depend on diffusion, it would have a lessened chance of survival when it is considered that the host actively absorbs this substance in competition. But since it appears that the tapeworm takes up glucose at a relatively faster rate than does the host (rat intestine, 34.7 micrograms/mg/hr at 1.6×10^{-2}M; and *H. diminuta,* 100 micrograms/mg/hr), one can possibly conclude that this tapeworm survives environmental conditions that could otherwise be quite hazardous.

SUMMARY

An extension of the study of glucose uptake by *Hymenolepis diminuta* supports the hypothesis that the process of permeation involved is an active one related to metabolic processes.

Iodoacetate at concentrations of 10^{-3}M and 3×10^{-4}M inhibits glucose uptake in 30-minute incubations; however, preincubation in iodoacetate was necessary in order to obtain inhibition of glucose uptake in 60 second incubations. The heating of worms reduced the glucose uptake rates to a level at which iodoacetate had no further depressing effect. DNP failed to inhibit the uptake of glucose by *H. diminuta* in 60-second incubation even after preincubation in the inhibitor. Sodium taurocholate inhibits glucose uptake by this worm in short interval experiments but only after preincubation in the taurocholate.

A gradient of uptake activity was demonstrated; the anteriormost portion of the worm was found to take up glucose at a higher rate than did the posterior segments of worm tissue.

The demonstration of apparent accumulation of glucose against a concentration gradient by *H. diminuta* was made in 60-second experiments. It was also shown

that, when placed in a salt solution favoring loss of glucose by diffusion, the worms lost no appreciable quantity of glucose.

Possible advantages of an active glucose uptake process in the economy of the tapeworm are discussed.

Literature Cited

Bacon, J. S. D. and Edelman, J. 1951 The carbohydrates of the Jerusalem artichoke and other compositiae. Biochem. J. **48:** 114–125.

Clark, G. A. 1922 Glucose absorption in the renal tubules of the frog. J. Physiol. **56:** 201–205.

Gustavson, B. E., Bergstrom, S., Lindstedt, S. and Norman, A. 1957 Turnover and nature of fecal bile acids in germ-free and infected rats fed cholic acid-24 C^{14} bile acids and steroids 41. Proc. Soc. Exp. Biol. Med. **94:** 467–471.

Höber, R. 1945 Physical Chemistry of Cells and Tissues. The Blakiston Co., Philadelphia.

Krane, SmM. and Crane, R. K. 1959 The accumulation of D-galactose against a concentration gradient by slices of rabbit kidney cortex. J. Biol. Chem. **234:** 211–216.

Nelson, N. 1944 A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153:** 375–380.

Phifer, K. 1959 Permeation and membrane transport in animal parasites: The absorption of glucose by *Hymenolepis diminuta*. J. Parasit. In press.

Phifer, K. 1960 Permeation and membrane transport in animal parasites: On the mechanism of glucose uptake by *Hymenolepis diminuta*. J. Parasit. In press.

Read, C. P., Schiller, E. L. and Phifer, K. 1958 The Role of Carbohydrates in the Biology of Cestodes. V. Comparative studies on the effects of host dietary carbohydrate on *Hymenolepis* species. Exp. Parasit. **7:** 198–216.

Rosenberg, T. 1954 The concept and definition of active transport. *In* Symposia of the Society for Experimental Biology **VIII:** 27–41.

Rothman, A. H. 1958 Role of Bile Salts in the Biology of Tapeworms. I. Effects on the metabolism of *Hymenolepis diminuta* and *Oochoristica symmetrica*. Exp. Parasit. **7:** 328–337.

Rothstein, A. and Meier, R. 1949 The Relationship of the Cell Surface to Metabolism. IV. The role of cell surface phosphatases of yeast. J. Cell. and Comp. Physiol. **34:** 97–114.

Smith, H. P., Groth, A. H. and Whipple, G. H. 1928 Bile salt metabolism. I. Control diets, methods and fasting output. J. Biol. Chem. **80:** 659–669.

Walker, A. M. and Hudson, C. L. 1937 The reabsorption of glucose from the renal tubule in amphibia and the action of phlorizin upon it. Am. J. Physiol. **118:** 130–143.

Wilbrandt, W. 1954 Secretion and transport in non-electrolytes. *In* Symposia of the Society for Experimental Biology **VIII:** 136–162.

——— and Laszt, L. 1933 Untersuchen über die Ursachen der selektiven Resorption der Zucker aus dem Darm. Biochem. Z. **259:** 398–417.

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: ON THE MECHANISM OF GLUCOSE UPTAKE BY *HYMENOLEPIS DIMINUTA**

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In recent studies (Phifer, 1959, 1960), it has been demonstrated that the tapeworm, *Hymenolepis diminuta,* takes up glucose against a concentration gradient by processes having characteristics which suggest coupling to energy metabolism. Observations on the effects of innate physiological factors also supported this hypothesis.

Since it appears that a catalytic mechanism is involved in the movement of glucose from the medium into the worm, it seemed important to test some of the hypotheses which have been put forward to account for glucose absorption in other tissues. The present inquiry may be broken down into three categories relating to (1) the effect of addition of an energy source on glucose uptake; (2) stereospecificity of the uptake of glucose; (3) the nature of systems involved.

MATERIALS AND METHODS

The cysticercoids of the tapeworm, *Hymenolepis diminuta,* were reared in *Tribolium confusum.* Each rat host was infected with ten cysticercoids. The worms were then allowed to grow to the definitive "adult" size (ca. 20 days).

The experimental methods used in this study of the effect of preincubation in an energy source and in the study of stereospecificity were those described previously as short interval experiments (Phifer, 1959, 1960). Worms were incubated for 60 seconds in uniformly labelled C^{14} glucose and the amount of labelled compound taken up by the worms was extracted at the termination of the incubation.

The assay of phosphatase activity which was a modification of that described by Erasmus (1957), depended upon the tapeworm's splitting the phosphate from the colorless compound, para-nitrophenyl phosphate, forming paranitrophenol which is yellow in color. The yellow color of the cleaved compound was measured in the Klett colorimeter using the 42 filter. The data presented here are expressed as micrograms of p-nitrophenol formed per gram of wet weight of worm tissue in 15 minutes. The incubations were carried out by adding each worm to a tube containing a buffered solution of the substrate in a volume of five ml containing substrate at the desired concentration and pH. The buffer used was 0.1 M Trishydroxymethylaminomethane. The incubations were performed in a 37° C. water bath for 15 minutes. In all of the experiments, except those in which the pH was varied, the pH was 9.5. The concentration of p-nitrophenyl phosphate in experiments in which the pH was varied was 3.5×10^{-3} M.

RESULTS

Transfer of a substance from a state of low chemical potential to that of higher chemical potential requires some sort of coupling with processes that release free energy. It has been shown that compounds known to inhibit energy metabolism

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tend to decrease glucose absorption by *H. diminuta* (Phifer, 1959, 1960). However, it might be argued (Rosenberg, 1954) that although changes in the rate of penetration can be due to changes in the driving forces, they could also be due to changes in membrane resistance which depends upon its structure. This structure might depend on such factors as concentration, temperature and the normal occurrence of enzyme reactions.

Therefore, a short interval experiment was designed in which this question could be examined. Worms were preincubated in Krebs-Ringer solution for equilibration purposes, after which six worms were placed in 10^{-3}M unlabelled glucose and six worms were placed in fresh Krebs-Ringer solution for an additional 30 minutes. The results of this experiment, presented in Table I, show that under

TABLE I. *The effect of preincubation in unlabelled monosaccharides on the uptake of C^{14} glucose by* H. diminuta *from unstarved and fasted hosts.*

Experimental variable	Uptake in CPM/mg	Number of worms
	Unstarved Hosts	
No preincubation	140.4 (25.1)	6
Preincubation in 10^{-3}M glucose	140.4 (31.2)	6
	Fasted Hosts	
No preincubation	153.9 (21.4)	8
Preincubation in 10^{-3}M glucose	212.2 (14.8)	9
	Fasted Hosts	
No preincubation	120.5 (14.1)	12
Preincubation in 10^{-3}M galactose	165.4 (25.9)	6
Preincubation in 10^{-3}M fructose	102.9 (13.7)	6

The values presented are means and their standard deviations.

these conditions, preincubation in glucose which was unlabelled had no appreciable accelerating effect upon the uptake of labelled glucose. The worms had been taken from unstarved rats.

Read and Rothman (1957) reported that starving the rat host brought about increased glucose utilization by *Hymenolepis diminuta,* indicating a fairly high level of endogenous glucose supply in the worm. Therefore, it was thought that if rats were starved for 48 hours and the worms from these rats were used in an experiment of the type mentioned above, an effect of preincubation in an energy source might be noticed. The results of this experiment, shown in Table I indicate that preincubation of starved worms in the presence of an energy source stimulates the mechanism of glucose transport, thus strengthening the hypothesis that energy metabolism is related to glucose transport in this animal.

An additional experiment was carried out in which two other monosaccharides were used for preincubation. One of these is metabolized by *H. diminuta* and one is not (Laurie, 1957). From Table I it can be seen that preincubation in galactose, which is utilized by this worm in glycolysis, increased the amount of C^{14} glucose taken up by the worms in 60 seconds This increase over the control value is significant at the 95 percent confidence level. On the other hand, preincubation in fructose, which is not metabolized by *H. diminuta,* did not increase the amount of C^{14} glucose absorbed by the worms. This experiment was carried out using worms from hosts deprived of food for 48 hours previous to the experiment.

If attainment of a fixed maximal rate of uptake is interpreted as due to a limited capacity of the uptake mechanism, competition among substances taken up by the same mechanism is to be expected (Wilbrandt, 1954). Cori (1926) showed that the amount absorbed from a mixture of two sugars was less than the sum of the

amounts absorbed from solutions of the single sugars in the same concentrations; in fact, it was only about equal to each of these amounts. However, stereospecificity of uptake must be distinguished from the problem of energetics of active transport of molecules across natural membranes (Cohen and Monod, 1957).

Experiments were carried out in which various sugars were incubated with glucose in order to see whether any could inhibit the uptake of glucose by *H. diminuta*. Thus it could be determined whether the glucose transport system was specific for glucose. The sugars tested were galactose, mannose, 3-methyl glucose, fructose, sorbose, xylose, 2-deoxyglucose and 2-deoxygalactose. Sodium glycerophosphate and glucosamine were also tested. In each experiment, six experimental animals as well as six control animals were incubated. The results of these experiments are summarized in Table II. The figures given are mean values. Each

TABLE II. *The effect of various sugars and sugar derivatives on the uptake of C^{14} glucose by* Hymenolepis diminuta.

Sugar	Mean uptake by control worms	Mean uptake by experimental worms
Galactose	118.3 (8.8)	97.4 (7.2)
Mannose	118.3 (8.8)	113.1 (12.7)
3-Methyl glucoside	84.3 (9.5)	67.5 (11.3)
Fructose	85.4 (11.1)	73.4 (14.0)
Sorbose	84.3 (12.8)	96.9 (19.0)
Xylose	108.4 (25.8)	89.7 (17.4)
Glucosamine	102.6 (14.4)	106.5 (15.9)
Glycerophosphate	117.7 (34.7)	89.9 (16.5)
2-Deoxyglucose	105.3 (16.2)	90.7 (18.3)
2-Deoxygalactose	62.4 (21.6)	68.7 (28.9)

Uptake is expressed in counts per minute per mg taken up in 60 seconds. Standard deviations of the means are in parentheses.

sugar was at a concentration twice that of glucose. In all cases but the galactose and mannose experiments, the concentration of glucose was 2×10^{-3}M; in the two exceptions, the glucose concentration was 10^{-3}M. Hence, under the conditions of these experiments, it can be seen that none of the sugars and sugar derivatives tested, interfere with the uptake of glucose by *Hymenolepis diminuta*. A possible conclusion might be that the site at which glucose enters the worm is highly specific for this particular sugar.

The view that phosphatases are involved in glucose absorption is based on (1) the hypothesis that glucose undergoes phosphorylation and dephosphorylation during absorption (Verzar and MacDougall, 1936) and (2) the fact that alkaline phosphatase is found in large amounts in those sites where glucose transport is known to occur.

In the present study, it was found that *Hymenolepis diminuta* hydrolyzes p-nitrophenyl phosphate, a colorless compound, liberating p-nitrophenol, a yellow compound. The worm is colored a brilliant yellow by this compound but this color disappears after a few rinses in balanced salt solution. Thus, it is thought that this action occurs in the surface of the worm.

In order to determine more exactly the optimal conditions for assaying phosphatase activity of the worms, studies were made of the effect of hydrogen ion concentration and the effect of substrate concentration on this activity. Preliminary experiments showed that activity below pH 8.5 was quite low. Figure 1 shows that the maximal activity occurs at about pH 9.5 and from Figure 2, one can estimate the approximate value of the apparent Michaelis constant: 3.2×10^{-3}M.

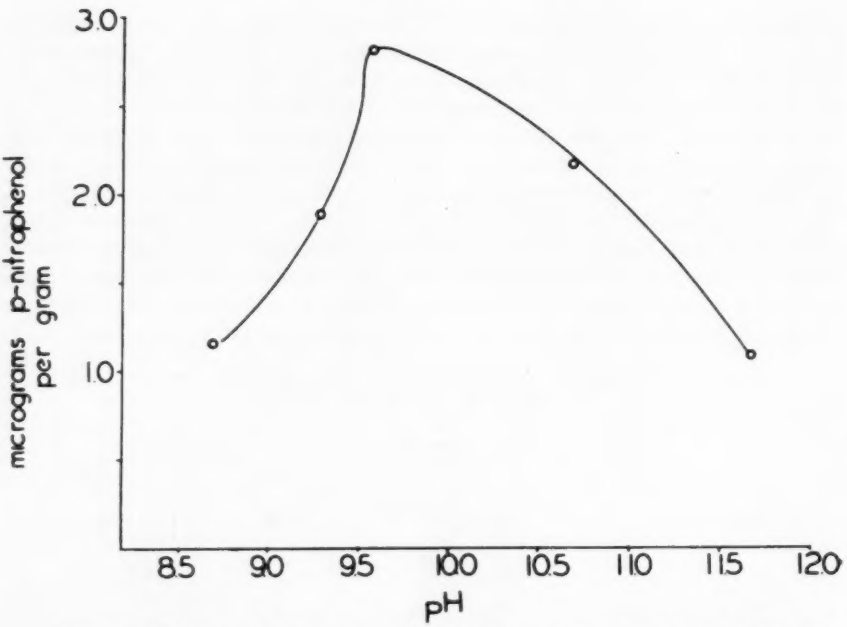

FIGURE 1. The effect of pH on the p-nitrophenyl phosphatase activity of *Hymenolepis diminuta*. The incubations were carried out for 15 minutes at 37° C. Each point is the average of 3 determinations.

Having shown that phlorizin inhibits the glucose absorption of *H. diminuta* (Phifer, 1959), it is of interest to compare this effect with that of phlorizin on the phosphatase activity of the worms. Animals were incubated in reaction mixtures containing (1) 3.3×10^{-3}M p-nitrophenyl phosphate and 2.6×10^{-4}M phlorizin; (2) 10^{-3}M p-nitrophenyl phosphate and 1.6×10^{-3}M phlorizin. In both cases, the concentration of substrate was below that which assures maximum velocity of the reaction, therefore making it possible for an inhibitor to act more readily. The results of these experiments can be seen in Table III. Thus, it can be seen that at concentrations much above the concentration of phlorizin which inhibits glucose absorption (2.1×10^{-4}M), there is no effect of phlorizin on this phosphatase system of *H. diminuta*.

Rothstein and Meier (1949), in studying the cell surface phosphatases of yeast, found that the most effective inhibitor tested was a molybdate salt. They also found that the concentration of molybdate used to inhibit phosphatase activity had no effect upon glucose consumption. Having seen (Phifer, 1960) that ammonium molybdate at a concentration of 6.5×10^{-4}M had no effect upon the glucose uptake by *H. diminuta*, experiments were designed to test the effect of this compound on the phosphatase system of this tapeworm. The data in Table IV show that, although the sample size was small, low concentrations of ammonium molybdate inhibit the phosphatase activity of *H. diminuta*.

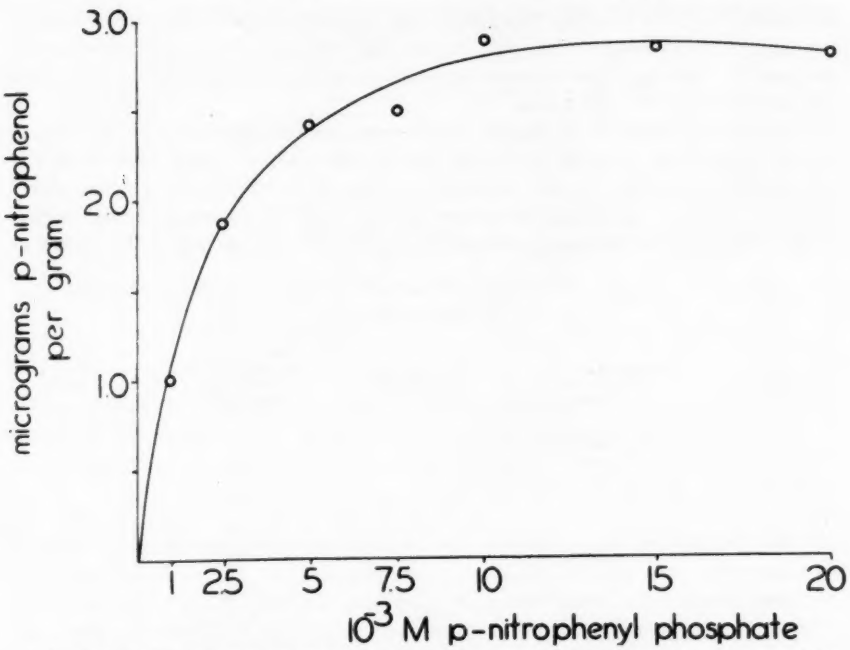

FIGURE 2. The effect of substrate concentration on the p-nitrophenyl phosphatase activity of *Hymenolepis diminuta*. The incubations were carried out for 15 minutes at 37° C. and pH 9.5. Each point is a mean value representing 3 determinations.

DISCUSSION

Although several metabolic inhibitors have been shown to reduce the level of glucose uptake by *Hymenolepis diminuta* (Phifer, 1959, 1960), these data do not constitute positive demonstration that metabolic energy is necessary for this tapeworm to transport glucose across its membranes, for Rosenberg (1954) has pointed out that changes in the rate of penetration brought about by metabolic inhibitors might be due to changes in membrane resistance occasioned by chemically induced structural changes. It is possible that these chemical compounds might denature surface proteins resulting in effects resembling those caused by heat-denaturation. Therefore, such data may be interpreted in more than one way.

On the other hand, it has been shown that, under certain conditions, preincubation in an energy source resulted in an increase in glucose uptake by the tapeworm. Worms from unstarved hosts were unaffected by preincubation in glucose, whereas worms from hosts which had been starved for 48 hours and were preincubated, took up glucose at a higher rate than did those which were not preincubated in glucose. The fact that preincubation in glucose and galactose increased the rate of glucose uptake whereas preincubation in fructose had no effect, is considered significant since this cestode is able to metabolize glucose and galactose but not fructose (Laurie, 1957). The data suggest the possibility that these compounds are taken up by the worm and undergo metabolic breakdown as a result of which energy is supplied to a carrier system. This is manifested by an additional "surge" of uptake activity. It

is obvious that this is highly speculative and contingent upon much more experimental evidence. However, the data suggest that one could profit from experiments designed to investigate the relationship of high energy phosphate compounds and glucose uptake in this tapeworm.

The fact that none of the sugars which were added to glucose in the incubation media reduced the level of the absorption of the glucose might mean that the mechanism responsible for the transport of glucose is specific for glucose alone. However, experiments should be carried out in which all the sugars tested in this series are studied to determine whether this worm will take up any of the sugars as

TABLE III. *The effect of phlorizin on the hydrolysis of p-nitrophenyl phosphate by* H. diminuta *in vitro.*

Experiment	Concentration of p-nitrophenyl phosphate	Concentration of phlorizin	Micrograms of nitrophenol formed per gram of wet weight	Mean (S.D.)
I	3.3×10^{-3}M	—	1.55	1.81 (0.35)
	"	—	2.06	
	"	2.6×10^{-4}M	1.66	1.60 (0.07)
	"	"	1.54	
II	3.3×10^{-3}M	—	1.16	1.36 (0.28)
	"	—	1.57	
	"	2.6×10^{-4}M	1.12	1.21 (0.11)
	"	"	1.29	
III	10^{-3}M	—	1.62	1.75 (0.17)
	"	—	1.87	
	"	1.6×10^{-3}M	1.82	
	"	"	2.26	2.14 (0.28)
	"	"	1.70	
IV	10^{-3}M	—	1.40	1.46 (0.08)
	"	—	1.51	
	"	1.6×10^{-3}M	1.59	
	"	"	1.54	1.57 (0.03)
	"	"	1.59	

readily as it takes up glucose. A true stereospecific site of permeation is indicative of a highly complex surface structure and one usually associated with an active transport process (Rosenberg, 1954).

One of the most commonly held hypotheses concerning glucose absorption is that of the intervention of a phosphorylation-dephosphorylation system. The emphasis has been mainly on dephosphorylation, specifically phosphatase systems. The basis of the hypothesis involves observations of the presence of phosphatase activity in those sites in which glucose transport is known to occur and the inhibition of glucose uptake by phlorizin which has been thought to influence phosphatase activity.

Phosphatases have been demonstrated to reside at the surface of certain tapeworms by Yamao (1952), Read (1950) and Erasmus (1957). Indeed, Erasmus has stated that since Read (1951) demonstrated a phosphorylase in the extracts of *H. diminuta* and that since phosphatases have also been demonstrated, the mech-

TABLE IV. *The effect of ammonium molybdate on the hydrolysis of p-nitrophenyl phosphate by* Hymenolepis diminuta.

Concentration of p-nitrophenyl phosphate	Concentration of Amonium molybdate	Micrograms of nitrophenol per gram of wet weight	Mean
5×10^{-4}M	—	0.73	
"	—	0.93	0.87
"	—	1.13	
"	—	0.71	
"	6.5×10^{-6}M	0.82	0.85
"	"	0.87	
"	6.5×10^{-5}M	0.29	
"	"	0.50	0.42
"	"	0.47	
"	6.5×10^{-4}M	0.038	0.041
"	"	0.045	

anism of glucose absorption may be one of phosphorylation with a secondary role played by the phosphatases. However, it must be emphasized that the demonstration of an enzyme in an extract and the existence of the enzyme at the neighborhood of the site of glucose absorption, both of which involve phosphate, may be completely distinct and distantly related phenomena.

A phosphatase, acting at the surface of *H. diminuta,* was shown to transform p-nitrophenyl phosphate to p-nitrophenol. The pH optimum was different from that observed by Read (1950) in a study of the hydrolysis of some other phosphate compounds by this tapeworm. Aside from possible experimental errors in this present study, this discrepancy might be due to the fact that different phosphatases were investigated. This is quite likely since the substrate in the present study was a phenyl derivative and unlike any of the compounds used by Read and since Read's studies were carried out with cell-free preparations.

It was shown that a concentration of ammonium molybdate which had no effect on glucose uptake by *H. diminuta* was very inhibitory toward this phosphatase system. This is an indirect indication that the two phenomena are not related in *H. diminuta.*

Gellhorn and Skupa (1933) and Riklis and Quastel (1958) found that potassium ion affected the permeability of the intestine to glucose so that, even at small concentrations of potassium, the uptake increased. The latter authors interpreted this potassium stimulation to be due to an acceleration of the rate of conversion of glucose to hexose-diphosphate prior to hydrolysis by intestinal phosphatase. The data presented previously (Phifer, 1959) indicated that potassium ion has no effect on the uptake of glucose by *H. diminuta.* Following the line of reasoning of Riklis and Quastel, perhaps this is an indication that glucose is not subjected to phosphorylation-dephosphorylation processes at the surface of the worm.

The data in Table III show that phlorizin at a concentration of 1.6×10^{-3}M had no effect on the phosphatase activity of *H. diminuta.* However, it was shown earlier that 2.1×10^{-4}M phlorizin inhibited the uptake of glucose in 60 seconds (Phifer, 1959), and since Laurie (1957) observed that brief washing of the worms caused a reversal of inhibition of glucose fermentation, it is indicated that this glucoside acts at the surface of the worm. The work of Crane, Field and Cori (1957) and of Lotspeich and Woronkow (1958) indicate that phlorizin blocks glucose transport at

a membrane site. The ready reversibility of inhibition and the fact that Lambrechts (1937) observed that methylation of phenolic hydroxyl groups inactivated phlorizin suggest that hydrogen bonding between the non-ionized phenolic hydroxyl groups of phlorizin and a locus on the surface of *H. diminuta* may be necessary in order to bring about inhibition. Davson and Danielli (1952) have pointed out that the formation of hydrogen bonds at a surface site tends to raise the energy barrier blocking permeation. A corollary experiment would be to test the effect of pH on the inhibition of glucose uptake by phlorizin.

SUMMARY

The study of glucose uptake by *Hymenolepis diminuta* was extended to include a consideration of possible mechanisms of transport. It was seen that if worms had been preincubated in either unlabelled glucose or galactose prior to incubation in C^{14} glucose, higher rates of uptake obtained than in cases in which preincubation in these sugars was omitted. Also, it was found that preincubation in fructose had no accelerating action.

The demonstration was made that the glucose uptake by *H. diminuta* is a relatively stereospecific process. None of the following sugars and sugar derivatives had any inhibitory action on glucose uptake when added to the incubation medium: galactose, mannose, sorbose, fructose, 2-deoxyglucose, 2-deoxygalactose, 3-methylglucose, glucosamine, xylose and glycerophosphate.

A surface p-nitrophenyl phophatase was demonstrated to occur in *H. diminuta* with a pH optimum of 9.5 and an apparent Michaelis constant of 3.2×10^{-3}M. It was shown that 1.6×10^{-3}M phlorizin had no effect on phosphatase activity whereas 6.5×10^{-5}M ammonium molybdate inhibited this phosphatase. These data were compared with the effects of molybdate and phlorizin on glucose uptake by *H. diminuta*.

The data are discussed in terms of their possible relation to mechanisms of glucose transport of this tapeworm.

LITERATURE CITED

COHEN, G. N. AND MONOD, J. 1957 Bacterial permeases. Bact. Rev. **21:** 169–194.

CORI, C. 1926 The rate of absorption of a mixture of glucose and galactose. Proc. Soc. Exp. Biol. Med. **23:** 290–291.

CRANE, R. K., FIELD, R. A. AND CORI, C. F. 1957 Studies of tissue permeability. I. The penetration of sugars into the Ehrlich ascites tumor cells. J. Biol. Chem. **224:** 649–662.

DAVSON, H. AND DANIELLI, J. F. 1952 The Permeability of Natural Membranes. Cambridge University Press, New York.

ERASMUS, D. 1957 Studies on phosphatase systems. I. Studies on *Taenia pisiformis* (cysticercus and adult). II. Studies on *Cysticercus tenuicollis* and *Moniezia expansa* (adult). Parasitology **47:** 71–91.

GELLHORN, E. AND SKUPA, A. 1933 The K-Ca antagonism in regard to absorption from the intestine. Am. J. Physiol. **106:** 318–322.

LAMBRECHTS, A. 1937 Nouvelles recherches sur le diabete phlorhizique, la phlorhizine et quelques substances apparentees. Arch. Intern. Physiol. **44** (supp.): 1–162.

LAURIE, J. 1957 The in vitro fermentation of carbohydrates by two species of cestodes and one species of acanthocephala. Exp. Parasit. **6:** 245–260.

LOTSPEICH, W. D. AND WORONKOW, S. 1958 Some quantitative studies on phlorizin inhibition of glucose transport in the kidney. Am. J. Physiol. **195:** 331–336.

PHIFER, K. 1959 Permeation and membrane transport in animal parasites: The absorption of glucose by *Hymenolepis diminuta*. J. Parasit. In press.

——— 1960 Permeation and membrane transport in animal parasites: Further observations on the uptake of glucose by *Hymenolepis diminuta*. J. Parasit. In press.

READ, C. P. 1950 Studies on the carbohydrate and related metabolism of the cestode, *Hymenolepis diminuta*. Dissertation, The Rice Institute.

——— 1951 Studies on the enzymes and intermediate products of carbohydrate degradation in the cestode *Hymenolepis diminuta*. Exp. Parasit. **1:** 1–18.

——— AND ROTHMAN, A. H. 1957 The Role of Carbohydrates in the Biology of Cestodes. II. The effect of starvation on glycogenesis and glucose consumption in *Hymenolepis*. Exp. Parasit. **6:** 280–287.

RIKLIS, E. AND QUASTEL, J. H. 1958 Effects of cations on sugar absorption by isolated surviving guinea pig intestine. Can. J. Biochem. and Physiol. **36:** 347–362.

ROSENBERG, T. 1954 The concept and definition of active transport. *In* Symposia of the Society for Experimental Biology **VIII:** 27–41.

ROTHSTEIN, A. AND MEIER, R. 1949 The relationship of the cell surface to metabolism. IV. The role of cell surface phosphatases of yeast. J. Cell. and Comp. Physiol. **34:** 97–114.

VERZAR, F. AND MACDOUGALL, E. J. 1936 Absorption from the Intestine. Longmans Green and Co., London.

WILBRANDT, W. 1954 Secretion and transport of non-electrolytes. *In* Symposia of the Society for Experimental Biology **VIII:** 136–162.

YAMAO, Y. 1952 Histochemical studies on endoparasites. VII. Distribution of the glycero-mono-phosphatases in the tissues of the cestodes, *Anoplocephala perfoliata, A. magna, Moniezia benedeni, M. expansa* and *Taenia taeniaeformis*. Zool. Mag. **61:** 254–260.

RESEARCH NOTE

MAINTENANCE AND REARING OF THE SNAIL *BULINUS TRUNCATUS* IN THE LABORATORY.

Successful rearing of the snail intermediate hosts of bilharziasis is an important aspect in the life cycle maintenance of the parasite in the laboratory. Most laboratories engaged in schistosome work use balanced aquaria of various sorts, together with snail diets of varying components of different substances. Some investigators, however, are satisfied with maintaining snail colonies in as simple and economical a manner as possible. Accordingly, it may be of interest to note the procedures used with *Bulinus truncatus* in connection with various laboratory studies during 1958, while with the WHO Bilharziasis Control Project in Iraq.

Bulinus was kept in non-aerated Baghdad tap water, which was changed daily. The volume varied from 30–150 ml per snail, depending on how many were kept together. In one experiment, where 30 snails were isolated individually in small dishes and followed for 33 days, the volume was 30 ml per snail. In another experiment 47 snails, in 4 groups of 10 to 17 each, were in rectangular aquaria with a volume of 150 ml per snail. Stock snails were kept in large circular aquaria, 100 ml per snail, from March through December in water temperatures from 15.5–25.5° C. No vegetation, mud, sand, or water additives of any kind were used, and in all cases the number of deaths was negligible.

The only dietary item was either boiled lettuce or spinach, depending on availability. The amount given was such that little or none remained at each daily water change. The amount is difficult to express quantitatively, but can be determined in a day or two by trial and error. It is important not to give excess food, since this results in the snails surfacing and crawling out of the aquaria and dishes. Glass covers are not required with large aquaria, but they are necessary for individual isolations in a small volume of water.

Egg-laying took place throughout the year and was almost exclusively on the glass surfaces, but occasionally on snail shells and bits of uneaten lettuce or spinach. Hatching of eggs occurred as early as 8 and as late as 19 days after oviposition in temperatures from 15.5–25.5° C. No difficulty was involved in rearing newly hatched snails on the same diet, and in tap water only.

By these procedures, snails between 1 and 2 mm long take between 7 and 8 weeks to commence reproduction.—HAIG H. NAJARIAN, *University of Texas Medical Branch, Galveston, Texas.*

RESEARCH NOTE

AN ANOMALY IN A PROGLOTTID OF *TAENIA PISIFORMIS* BLOCH, 1782.

While slides in the teaching collection of the Biology Department laboratories at New Mexico State University were being checked, a mounted specimen of tapeworm proglottids was found in which an anomalous condition existed. The label indicated that the specimen had been remounted by a student assistant in 1936, and there was no indication as to the original source of the material, i.e., whether it had been mounted from specimens collected locally or as a slide from a supply company. Photographic images of the proglottid were made by microprojection upon sensitized paper. The slide was then demounted and serial sections were made. The relationships of the ducts were reconstructed from the sections, and the drawings superimposed upon the photographic image. The accompanying drawing (fig. 1) is a tracing made from this modified photograph.

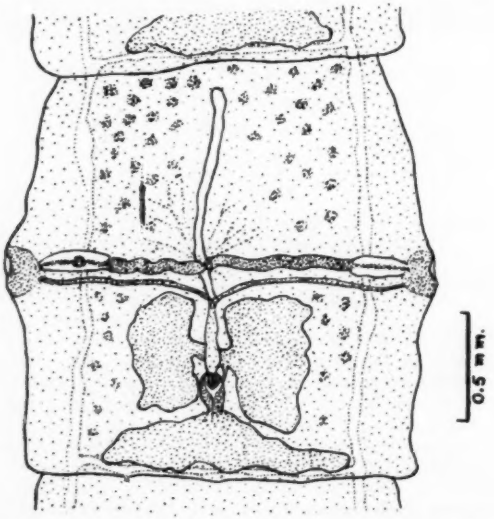

FIGURE 1. Anomalous double genital ducts in *Taenia pisiformis.*

The entire specimen consists of 3 proglottids, 2 of which are in every respect characteristic of *Taenia pisiformis.* The middle one, however, possesses 2 genital atria, 2 complete sets of male ducts, and 2 vaginae. The bilateral arrangement of the ducts and the atria will be noted in the figure. The 2 vasa deferentia appear to be continuous, uniting in the median line. Their lumina, however, are distinct, with the membranes of the 2 walls forming a double boundary mediadly. The vasa efferentia are about equally distributed, some from each side contributing to each vas deferens. Both vaginae enter a single receptaculum seminis. There is nothing to indicate that either of the sets of ducts have been non-functional.

The remainder of the structures are normal in every respect. The receptaculum seminis, fertilization canal, oötype and shell gland, oviduct, ovary, vitelline duct, vitellarium, and uterus are each single and their connections are in the usual pattern.—MARLOWE G. ANDERSON, *Department of Biology, New Mexico State University, University Park, New Mexico.*

STUDIES ON THE PENETRATION OF SCHISTOSOME CERCARIAE. I. ACTION OF THE ANTIHISTAMINE PROMETHAZINE HYDROCHLORIDE*

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The mechanism of penetration of schistosome cercariae has received some attention in recent years. It appears to be a combination of mechanical movement and chemical effects of glandular secretions from the cercariae which presumably allow them to enter the dermal blood vessels (Gordon and Griffiths, 1951; Griffiths, 1953; Standen, 1953; Stirewalt, 1956). Attempts to identify the secretions associated with the penetration glands have met with varied success. Several workers have reported the presence of hyaluronidase in cercariae of *Schistosoma mansoni* (Levine, Garzoli, Kuntz and Killough, 1948; Stirewalt and Evans, 1952; Kuntz, 1953; Evans, 1953), whereas others (Gordon and Griffiths, 1951; Lee and Lewert, 1957) have failed to demonstrate this enzyme. Lee and Lewert (1957) also were unable to show activity of extracts of *S. mansoni* cercariae on chondroitin sulfuric acid; however, they did demonstrate the presence of heparinase activity in these cercariae. Lewert and Lee (1954, 1956) and Millemann and Thonard (1959) showed that the cercariae of *S. mansoni* and *Schistosomatium douthitti* possess proteases.

Lewert and Lee (1954) observed that if Evans blue is administered intravenously into rats just before the cercariae of *S. mansoni* and *S. douthitti* are placed on the shaved skin, blue spots appeared within 2 to 3 minutes at the site of penetration. This bluing is similar to that which occurs when histamine is injected into the skin following intravenous injection of various dyes (Miles and Miles, 1952). The histamine bluing, however, may be completely inhibited by various antihistamines, whereas Lewert and Lee (1954) found that the Evans blue response produced by the larvae of *Strongyloides simiae* and *S. ratti* was not affected by antihistamines. They did not study the effect of antihistamines on the Evans blue response to penetrating schistosome cercariae.

One of us (S.E.M.), in connection with other experiments, has observed that if Evans blue is administered intravenously into rabbits, the accumulation of the dye at the intradermal sites of injection of histamine and bull testicular hyaluronidase is inhibited by prior administration of the antihistamine promethazine hydrochloride (Phenergan®). In view of these observations, it was decided to investigate the action of this compound on the Evans blue response to penetrating schistosome cercariae as well as its possible effect on the establishment of the parasites in laboratory animals. A study of this nature might aid in elucidating the mechanism of cercarial penetration and is the subject of this paper.

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MATERIALS AND METHODS

Cercariae of *S. douthitti* were collected from *Lymnaea stagnalis* snails raised and infected in the laboratory, and those of *S. mansoni* from laboratory raised and infected *Australorbis glabratus* snails. In both cases, cercariae were collected after normal emergence from 30 or more bisexually infected snails.

Rabbits of both sexes weighing from 2.4 to 3.2 kg and grouped according to weight and sex were infected percutaneously with 300 to 1,500 cercariae of either *S. mansoni* or *S. douthitti*, except in one experiment in which the cercariae of *S. mansoni* were injected into the marginal ear vein. The cercariae were placed on the shaved abdominal skin of the rabbit and left there for 60 minutes, after which the skin was allowed to dry. The cercariae were localized on the skin by placing them in the center of a rubber ring 3 cm in diameter which was cemented in place.

Swiss-Webster female mice, weighing from 20 to 35 g and grouped according to weight were infected percutaneously with 50 cercariae of either species of schistosome. In the case of *S. mansoni*, a modification of the method of Pan et al (1951) was used for exposing the animals. The abdominal skin was shaved with animal clippers and the mice were pinned out on plastic boards by means of adhesive tape placed around the legs so that the abdomen of the mouse lay in a center well of the board. The *S. mansoni* cercarial suspension was placed in the center well of the board and the mice left in place for 60 minutes, following which they were dried under a lamp. Mice were infected with *S. douthitti* by pinning them out on boards, abdominal side up, and placing the cercariae on the shaved surface by means of a wire loop (Kagan, Short and Nez, 1954). The skin was kept moist for 30 minutes and then dried under a lamp.

After 5 cc of a 0.5 percent solution of Evans blue was injected into the marginal ear vein of the rabbits, 0.25 cc of a 1 to 1000 dilution of histamine and 0.25 cc of saline solution containing 75 turbidity reducing units (TRU) of bull testicular hyaluronidase (Armour) were injected intradermally at separate sites on the shaved abdominal surface of the rabbit. The Evans blue, hyaluronidase, and histamine were injected approximately 5 minutes before the application of the cercariae.

Thirty minutes before application of the cercariae, each rabbit in the experimental group was injected subcutaneously with 2 cc of promethazine hydrochloride (Wyeth Laboratories) solution containing 50 mg promethazine hydrochloride, 4.5 mg sodium citrate, 1.5 mg citric acid, 2 mg sodium formaldehyde sulfoxylate, and 10.0 mg phenol. In some experiments the test rabbit received the same amount of pure crystalline promethazine hydrochloride (Wyeth Laboratories) made up in solution as previously outlined, but without the phenol and formaldehyde in order to eliminate any toxicity to the cercariae attributable to the latter compounds. Subsequent to the initial injection, the rabbits received 4 subcutaneous injections, except animal No. 1 which received 3 injections, of the promethazine hydrochloride solution at approximately 6- to 17-hour intervals. Control rabbits received either no injections or were injected in the same way as the experimental group, except that they received a placebo injection of a solution containing formaldehyde and phenol without promethazine hydrochloride. Promethazine hydrochloride excretion in the urine was determined in one rabbit according to the method of Forrest et al (1958).

In all instances the mice received only one injection of promethazine hydrochloride intraperitoneally 30 minutes before exposure to the cercariae. The experimental mice received either of the 2 solutions of the promethazine hydrochloride as described above, and the controls were uninjected or were injected with the placebo solution. In the initial experiments the mice received either 2.5 or 4.0 mg of promethazine hydrochloride. In later experiments the dosage was determined according to the weight of the animal based on an LD_{50} of 190 mg per kg of body weight for intraperitoneal injection (Spector, 1956).

Rabbits were autopsied 11 to 13 weeks after exposure to the cercariae and the adult worms were recovered using the technique of Yolles et al (1947). Mice were autopsied 6 to 9 weeks after exposure and the worms recovered by the perfusion method of Pan and Hunter (1951). After perfusion the mesentery was examined under the dissecting microscope for any remaining worms. Also, the liver was teased apart and examined for adult parasites.

RESULTS

Evans blue response. Ten rabbits were exposed to the cercariae of *S. mansoni* and 2 rabbits to the cercariae of *S. douthitti*. The experimental animals received the promethazine hydrochloride either with or without (Nos. 3, 6, 9) formaldehyde and phenol as already described. The control rabbits were either uninjected or received a placebo injection of formaldehyde and phenol. The results are summarized in table I and depicted in figures 1 and 2. In all the control rabbits Evans blue

TABLE I. *Evans blue response in rabbits exposed to the cercariae of* Schistosoma mansoni *and* Schistosomatium douthitti *following subcutaneous injection of promethazine hydrochloride.*

No. Rabbit	Promethazine hydrochloride	No. cercariae of *S. mansoni*	Number of of worms recovered at autopsy	Evans blue response at site of cercarial penetration
1	Yes	300–400	89	Few spots—appeared at 40 min.
2	No	"	112	Intense—at 10 min.
3	Yes	1,000–1,500	251	Few spots at 20 min., slight increase in intensity
4	No	"	380	Intense at 10 min.
5	No	"	334	" " " "
6	Yes	300–400	7	Few spots at 30 min.
7	No	"	134	Intense at 10 min.
8	Yes	"	73	No spots
9	Yes	"	82	" "
10	No	"	0	Intense at 10 min.
		No. cercariae of *S. douthitti*		
1	Yes	400	1	Few spots at 20 min.
2	No	"	324	Intense at 10 min.

accumulated at the sites of injection of the histamine and hyaluronidase. The dye reaction began approximately 15 minutes following the injection of these substances, reached its maximum diameter and color intensity 30 minutes following injection, and persisted for over an hour. In a similar manner the dye accumulated at the site of cercarial penetration beginning approximately 10 minutes following application of the cercariae and reached its maximum diameter and color intensity 20 minutes following exposure. The reaction persisted for over an hour after which it was no longer followed. Larger numbers of cercariae produced a more intense reaction. Dye also accumulated at the site of cercarial penetration in rabbit No. 5 receiving the placebo injection, indicating that the vehicle for the promethazine hydrochloride had no effect on the response.

In the experimental rabbits receiving either of the promethazine hydrochloride solutions, no dye accumulated at the site of injection of the hyaluronidase. A few small pinpoint spots of dye appeared at the site of injection of the histamine and at the site of cercarial penetration. In both instances, the dye appeared about 30 minutes after the application of the cercariae or the injection of the histamine, and never reached the intensity seen in the experimental rabbits. The small accumulation of dye suggests that some cercariae were successful in penetrating the skin and that the antihistamine at the concentration used served only to delay the penetration of these cercariae in addition to preventing many cercariae from penetrating at all. That some cercariae were successful in penetrating was shown by the finding that all rabbits except No. 10 were infected; however, in most cases but not all, the experimental animals had fewer adult worms than the controls.

Rabbit infection experiments. In order to confirm the findings in the Evans blue experiments that promethazine hydrochloride partially inhibits the penetration of schistosome cercariae, 6 rabbits were exposed to the cercariae of *S. mansoni* and 3 rabbits to the cercariae of *S. douthitti.* The results are presented in table II. As expected, those animals receiving the antihistamine had considerably fewer adult worms at autopsy than the control rabbits, except in the case of Nos. 5 and 6.

A preliminary experiment was performed to determine if the action of the antihistamine was on cercarial penetration or on the subsequent maturation of the worms once they had attained the circulatory system. Two rabbits received the promethazine hydrochloride and 2 served as uninjected controls. Approximately 500–1000 cercariae of *S. mansoni* were injected into the marginal ear vein of the

TABLE II. *The effect of promethazine hydrochloride on the infection of rabbits with* S. mansoni *and* S. douthitti.

Rabbit No.	Promethazine hydrochloride	No. cercariae of *S. mansoni*	No. of worms recovered at autopsy
1	Yes	400–500	16
2	Yes	"	78
3	No	"	436
4	No	"	139
5	Yes	300–1,000	75
6	No	"	54
		No. cercariae of *S. douthitti*	
1	Yes	500	81
2	No	500	145
3	No	400	117

animals. Unfortunately, the experiment was unsuccessful in that 3 animals, including both controls, were uninfected at autopsy and the remaining rabbit yielded only 8 worms. Moore et al (1949) have shown that animals may be infected by other than the percutaneous route. These investigators infected rabbits by intraperitoneal injection of the cercariae of *S. mansoni,* although this method was not as efficient as percutaneous exposure. In view of these observations, it would seem that animals could be infected by placing cercariae directly into the circulatory system. Failure to obtain infection by this method in the above experiment may be due solely to faulty technique. This approach is one way of determining at what stage of infection the antihistamine is exerting its action and is under further investigation.

Mouse infection experiments. Mice were exposed, as previously described, to 50 cercariae of *S. mansoni* or *S. douthitti.* The experimental animals received a single injection of either of the promethazine hydrochloride solutions and the control animals were either uninjected or received a placebo injection. The results are summarized in table III. Four experiments were carried out with *S. mansoni* and 3 with *S. douthitti.* The test and control groups of mice in each experiment consisted initially of 8 to 11 animals. Four mg of promethazine hydrochloride was the usual dosage except in experiment 1, in which one group of mice received

TABLE III. *The effect of promethazine hydrochloride on the infection of mice with* S. mansoni *and* S. douthitti.

Exp. No.	Promethazine hydrochloride (mg)	Number of mice	Number of worms recovered at autopsy		Number of mice uninfected
			Av. per animal	(Range of group)	
S. mansoni					
7	2.5	7	37	(11–63)	0
	4.0	9	8.8	(0–32)	4
	None	8	51.6	(25–85)	0
11	4.0	8	12	(0–20)	2
	4.0	7	15	(0–51)	3
	None	9	36	(14–52)	0
	None (placebo injection)	10	27	(8–44)	0
12	4.0	11	14	(22–45)	6
	None	11	41	(17–74)	0
14	Dosage based on body wt.	4	0.25	(0–1)	3
	None	7	27	(18–39)	0
S. douthitti					
10	4.0	7	0.4	(0–2)	5
	None	8	All died—not examined		
13	4.0	8	33	(26–43)	0
	4.0	6	25	(23–28)	0
	None	3	21	(15–27)	0
	None (placebo injection)	4	28	(19–35)	0
15	Dosage based on body wt.	2	17	(14–20)	0
	None	5	35	(26–45)	0

2.5 mg and in experiments 14 and 15 in which the dosage was based on body weight. Deaths always occurred in the first few days among those mice receiving the antihistamine, and took from 1 to 8 animals per group. In the case of *S. mansoni* there were always far fewer adult worms recovered at autopsy in the experimental than in the control groups. Moreover, the pathological picture, which is of course a reflection of the worm burden, was always less severe in the former group, and there were always several animals in this group that were uninfected, whereas all mice in the control groups were infected. The number of worms recovered at autopsy appears to be directly related to the dosage of the antihistamine as shown in experiment 7. In experiment 11 the mice receiving the placebo injection had significantly more worms than either of the 2 test groups and almost as many as the control group, indicating, as shown previously in the rabbit experiments, that the formaldehyde and phenol had no effect on the penetration and subsequent establishment of the worms. Both of the promethazine hydrochloride solutions were equivalent in their action on the parasites, as shown in experiment 11. The second group of mice in this experiment received the drug in a solution not containing formaldehyde and phenol. These results indicate that the inhibitory effect can be attributed to the antihistamine and not to the vehicle in which it was dissolved.

The effect of the drug on *S. douthitti* was not as marked as on *S. mansoni*. The results of experiments 10 and 15 suggest, however, that the antihistamine also is effective in inhibiting the infection of mice with *S. douthitti*. In experiment 10, five of the 7 test animals were uninfected and the remaining 2 mice had 1 and 2 worms at autopsy. Unfortunately, the control animals in this experiment died and were not available for examination. These animals, however, died 30 to 40 days following exposure to the cercariae and in our experience this is the time of death of mice after exposure to 50 cercariae of *S. douthitti*. Moreover, we have never failed to infect mice with this parasite. The data, although inadequate, indicate that the test animals were protected by the drug. The results of experiment 15, in which the dosage of the antihistamine was based on body weight, lend further support to the interpretation that the drug exerts an inhibitory action on the penetration and/or maturation of the parasites. As in previous experiments, many of the mice died of the toxicity of the drug. The 2 survivors, however, had significantly fewer worms than the controls, and the pathological changes (pseudotubercle formation in the liver and spleen, and hemorrhage into the intestine) were considerably less severe than those seen in the control animals. The results of experiment 13 seem to indicate that both antihistamine solutions were not effective in preventing infection. The second group of mice in this experiment received the drug in a solution not containing formaldehyde and phenol. An explanation for these apparent contradictory results is not evident from the available data. It may be that *S. douthitti* is less sensitive to the action of the antihistamine at the dosage employed than is *S. mansoni*. Experiments bearing on this question are in progress and will be reported at a later date.

DISCUSSION

The accumulation of Evans blue dye at the site of cercarial penetration was observed by Lewert and Lee (1954). Our studies have confirmed these observations. The Evans blue response to penetrating cercariae is grossly similar to that

observed when histamine or hyaluronidase is injected into the skin, as shown in the present study. However, Lewert and Lee (1954) have demonstrated that a second application of *Strongyloides ratti* larvae to the same skin area again results in the production of blue spots, whereas with histamine Miles and Miles (1952) have shown that a previously injected area became refractory and did not respond to a second injection. No attempt was made in the present study to determine if a second application of cercariae would again result in the production of blue spots.

It is known that the extravasation of a dye injected intravenously does not necessarily indicate the presence of hyaluronidase in the test material, in our case living cercariae. In fact, Benditt et al (1951) have shown that purification of bull-testis hyaluronidase tends to render it incapable of increasing capillary permeability and that the impurities in the extract are actually responsible for the observed increase in vascular permeability. In the present study the accumulation of dye at the site of injection of the hyaluronidase may be due to such impurities. Furthermore, the gross similarity between the cercarial and hyaluronidase bluing is not taken to mean that these parasites possess this enzyme. This is especially so in view of the fact that Lee and Lewert (1957) and the present writers (unpublished observations) have not been able to demonstrate this enzyme in cercarial extracts by means of turbidimetric and colorimetric tests. Stirewalt and Evans (1952) and Evans (1953), however, claim to have demonstrated this enzyme in cercariae on the basis of the streptococcal decapsulation test. The observation in the present study that the hyaluronidase bluing is inhibited by an antihistamine is interesting and to our knowledge has not been described previously.

Our finding that the cercarial bluing is inhibited by an antihistamine is in contrast to the findings of Lewert and Lee (1954) that the antihistamines neoantergan and benadryl hydrochloride do not prevent the accumulation of dye at the site of penetration of *Strongyloides ratti* and *S. simiae* larvae. We have not yet tested these compounds on schistosome cercariae. The differences in the action of the antihistamines may be related to different mechanisms of penetration of these various parasites, or simply due to differences in the pharmacological properties of these drugs.

It is clear from our experiments that the antihistamine promethazine hydrochloride inhibits to a large extent the penetration and/or maturation of both species of schistosomes in rabbits and in mice. The fact that most of the rabbits and some of the mice were infected may be related in part to the dosage of the antihistamine and to the number of cercariae used in our studies. The observations of Schmiterlow (1956) may also be pertinent in this regard. He has shown that promethazine disappears from the blood of mice within 1 hour following intramuscular injections and reaches a high concentration in the tissues, particularly the lungs and then disappears from the body within 24 hours. These observations suggest that the antihistamine may be acting at the time when the cercariae are migrating in the skin, and perhaps also when they are passing through the pulmonary tissue. Furthermore, in view of the relatively rapid excretion of this compound by the animal, any viable cercariae remaining in the skin after this time may then be capable of initiating an infection. This interpretaton receives further support from the studies of Yolles et al (1949) who demonstrated that the cercariae of *S. mansoni* can be found in the skin of rabbits for as long as 9 days after exposure and possibly

for as long as 14 days. The excretion of this drug by the rabbit was also relatively rapid with the dosage employed by us. In 1 rabbit the excretion of the antihistamine began 5 hours after the first injection and continued for 36 hours after the last injection; thereafter, the drug could no longer be detected in the urine.

The mechanism of action of the antihistamine on the infection of animals with these parasites cannot be stated satisfactorily at the present time. In the light of the above observations, we feel that the increase in capillary permeability, as indicated in the Evans blue experiments, may be mediated through the release of histamine brought about by the action of the cercarial enzymes. These enzymes, as shown by Lewert and Lee (1954), are directed against the glycoproteins of the basement membrane and ground substance, and perhaps against the glycoprotein constituents of the capillaries. This enzymatic activity may result in an alteration in capillary permeability, as suggested by the above authors, and, as we propose, possibly in the release of histamine either directly or indirectly from mast cells concentrated around the blood vessels or in the tissue spaces. The alteration in capillary permeability may aid the cercariae in gaining access to the circulation. These reactions could be inhibited by the presence of the antihistamine which in turn might be expected to concentrate in the area where the capillary permeability is altered. Such an interpretation receives further support from the fact that promethazine has an exceptional ability to restore the permeability of capillaries (Paton, 1957). Moreover, if histamine is involved in the penetration of cercariae, it is possible that a temporary exhaustion of the available histamine occurs following penetration. The exhaustion of the histamine might result, then, in the development of a short-lived local skin "immunity" as observed by Stirewalt (1953). Experiments bearing on these theoretical considerations are now in progress and will be reported in a subsequent publication.

SUMMARY

Evans blue dye injected intravenously into rabbits accumulated in the skin at the site of penetration of *Schistosoma mansoni* and *Schistosomatium douthitti* cercariae. The dye also accumulated at the intradermal sites of injection of histamine and hyaluronidase. Promethazine hydrochloride, an antihistamine, inhibited the Evans blue response to the cercariae as well as to the histamine and the hyaluronidase. These results are in contrast to those of others in which other antihistamines did not prevent the concentration of dye at the site of penetration of *Strongyloides* larvae.

The antihistamine also inhibited to a large extent the infection of rabbits and mice with *S. mansoni* and *S. douthitti*. An explanation is offered for the possible mechanism of action of the drug with respect to its inhibitory action on the parasites.

These observations are discussed in terms of the mechanism of penetration of schistosome cercariae.

LITERATURE CITED

BENDITT, E. P., SCHILLER, S., MATHEWS, M. B. AND DORFMAN, A. 1951 Evidence that hyaluronidase is not the factor in testicular extract causing increased vascular permeability. Proc. Soc. Exp. Biol. Med. **77**: 643–646.

EVANS, A. S. 1953 Quantitative demonstration of hyaluronidase activity in cercariae of *Schistosoma mansoni* by the streptococcal decapsulation test. Exp. Parasit. **2**: 417–427.

FORREST, F. M., FORREST, I. S. AND MASON, A. S. 1958 A rapid urinary test for chlorpromazine, promazine and pacatal: a supplementary report. Am. J. Psychiatry **114:** 931–932.

GORDON, R. M. AND GRIFFITHS, R. B. 1951 Observations on the means by which the cercariae of *Schistosoma mansoni* penetrate mammalian skin, together with an account of certain morphological changes observed in the newly penetrated larvae. Ann. Trop. Med. Parasit. **45:** 227–243.

GRIFFITHS, R. B. 1953 Further observations on the penetration of mammalian skin by the cercariae of *Schistosoma mansoni,* with special reference to the effects of mass invasion. Ann. Trop. Med. Parasit. **47:** 86–94.

KAGAN, I. G., SHORT, R. B. AND NEZ, M. M. 1954 Maintenance of *Schistosomatium douthitti* (Cort, 1914) in the laboratory (Trematoda: Schistosomatidae). J. Parasit. **40:** 1–16.

KUNTZ, R. E. 1953 Demonstration of the "spreading factor" in the cercariae of *Schistosoma mansoni.* Exp. Parasit. **2:** 397–402.

LEE, C. L. AND LEWERT, R. M. 1957 Studies on the presence of mucopolysaccharidase in penetrating helminth larvae. J. Inf. Dis. **101:** 287–294.

LEVINE, M. D., GARZOLI, R. F., KUNTZ, R. E. AND KILLOUGH, J. H. 1948 On the demonstration of hyaluronidase in cercariae of *Schistosoma mansoni.* J. Parasit. **34:** 158–161.

LEWERT, R. M. AND LEE, C. L. 1954 Studies on the passage of helminth larvae through host tissues. I. Histochemical studies on extracellular changes caused by penetrating larvae. II. Enzymatic activity of larvae in vitro and in vivo. J. Inf. Dis. **95:** 13–51.

——— AND ——— 1956 Quantitative studies of the collagenase-like enzymes of cercariae of *Schistosoma mansoni* and the larvae of *Strongyloides ratti.* J. Inf. Dis. **99:** 1–14.

MILLEMANN, R. E. AND THONARD, J. C. 1959 Protease activity in schistosome cercariae. Exp. Parasit. **8:** 129–136.

MILES, A. A. AND MILES, E. M. 1952 Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs. J. Physiol. **118:** 228–257.

MOORE, D. V., YOLLES, T. K. AND MELENEY, H. E. 1949 A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni.* J. Parasit. **35:** 156–170.

PAN, C. AND HUNTER, G. W. III. 1951 A modified perfusion technique for the recovery of schistosomes. J. Lab. Clin. Med. **37:** 815–816.

———, KAUFMAN, E. H. AND HUNTER, G. W. III. 1951 A technique for infecting small mammals with *Schistosoma japonicum.* J. Lab. Clin. Med. **37:** 817–819.

PATON, W. D. M. 1957 Histamine release by compounds of simple chemical structure. Pharm. Rev. **9:** 269–328.

SPECTOR, W. S. 1956 Handbook of Biological Data. W. B. Saunders Co., Philadelphia.

SCHMITERLOW, C. G. 1956 An autoradiographical study of the distribution of S 35-labelled promethazine in the body. Arch. Internatl. Pharmacodynamie et Ther. **108:** 431–435.

STANDEN, O. D. 1953 The penetration of the cercariae of *Schistosoma mansoni* into the skin and lymphatics of the mouse. Trans. Roy. Soc. Trop. Med. Hyg. **47:** 292–298.

STIREWALT, M. A. 1953 The influence of previous infection of mice with *Schistosoma mansoni* on a challenging infection with the homologous parasite. Am. J. Trop. Med. Hyg. **2:** 867–882.

——— 1956 Penetration of host skin by cercariae of *Schistosoma mansoni.* I. Observed entry into the skin of mouse, hamster, rat, monkey and man. J. Parasit. **42:** 565–580.

——— AND EVANS, A. S. 1952 Demonstration of an enzymatic factor in cercariae of *Schistosoma mansoni* by the streptococcal decapsulation test. J. Inf. Dis. **91:** 191–197.

YOLLES, T. K., MOORE, D. V., DEGIUSTI, D. L., RIPSOM, C. A. AND MELENEY, H. E. 1947 A technique for the perfusion of laboratory animals for the recovery of schistosomes. J. Parasit. **33:** 419–426.

———, ——— AND MELENEY, H. E. 1949 Post-cercarial development of *Schistosoma mansoni* in the rabbit and hamster after intraperitoneal and percutaneous infection. J. Parasit. **35:** 276–294.

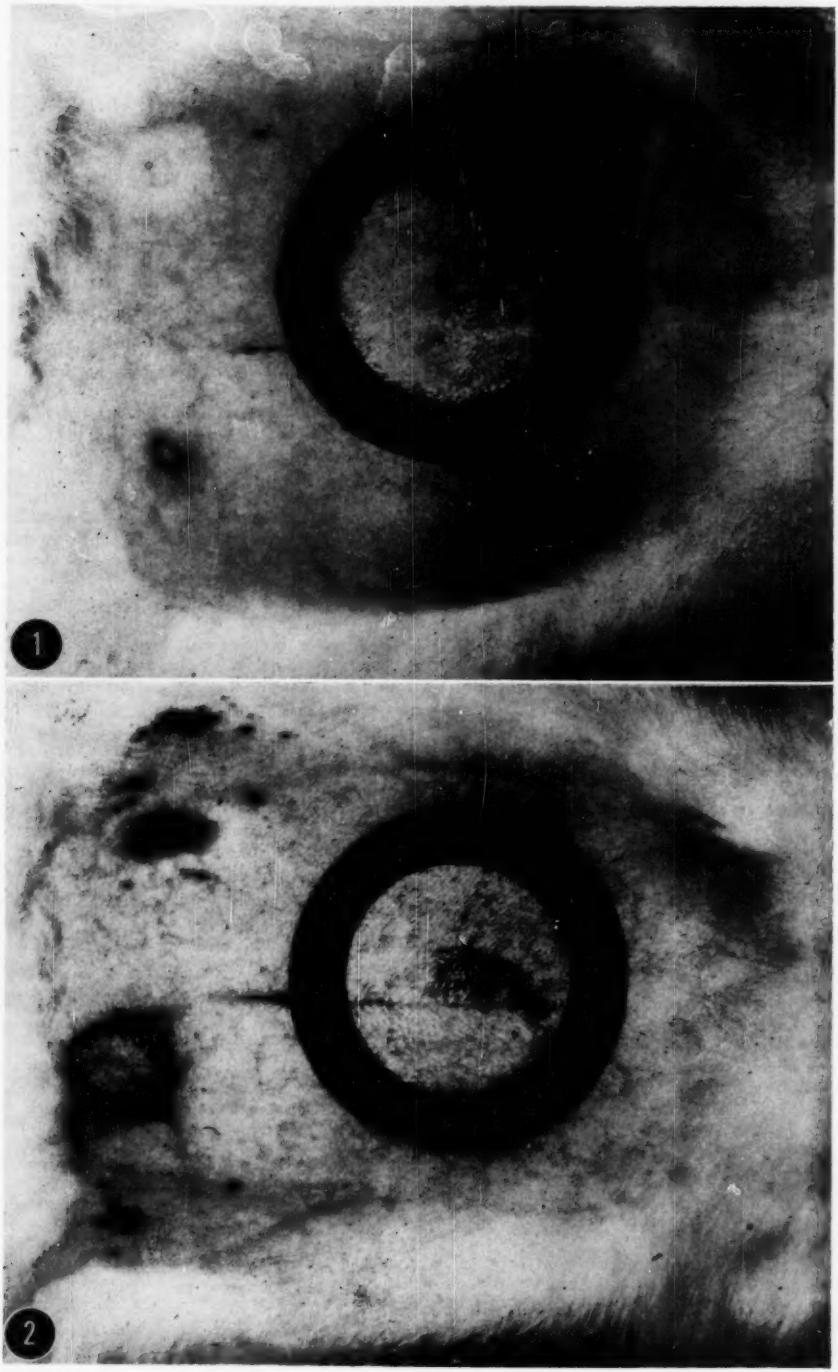
EXPLANATION OF PLATE I

Evans blue response in the skin of rabbits to *S. mansoni* cercariae, histamine and hyaluronidase. The pictures were taken 30 minutes after exposure to the cercariae.

FIGURE 1. Animal injected with 50 mg of promethazine hydrochloride subcutaneously 30 minutes before exposure to cercariae; dye not accumulated in the skin at sites of cercarial penetration or at hyaluronidase site (upper left corner); slight accumulation of dye at histamine site (lower left corner).

FIGURE 2. Uninjected control animal; dye accumulated at sites of cercarial penetration and at the hyaluronidase and histamine sites.

PLATE I



RESEARCH NOTE

HOLOTRICH CILIATES ENTOCOMMENSAL IN THE SEA URCHIN *STRONGYLOCENTROTUS ECHINOIDES* FROM SAN JUAN COUNTY, WASHINGTON.

The entocommensal ciliate fauna of echinoids inhabiting the Pacific coast of North America is poorly known in comparison with that of the Atlantic, Caribbean and western Pacific waters. The classical investigations of Lynch (1929–30, Univ. Cal. Publ. Zool. **33**: 27–56, 307–350) on species resident in the intestinal tracts of the sea urchins *Strongylocentrotus purpuratus* (Stimpson) and *S. franciscanus* A. Agassiz represent the sole contributions from this region. The recent revival of interest in this unique microcommunity of ciliates by several Russian investigators, notably Poljansky and Strelkov, warrants attention. Since 1951, these workers have described forms from *Strongylocentrotus* spp. of the northwestern Pacific Ocean in several articles in *Zoologitsheskii Zhurnal* and *Parazitologicheskii sbornik*.

In August 1959 several dredge hauls were made from the Hydah, research vessel of the Friday Harbor Laboratories of the University of Washington, at various depths in the sublittoral zone of President Channel (vicinity of latitude 48° 40′ N; longitude 123° W.), San Juan County, Washington. They yielded numerous specimens of *Strongylocentrotus echinoides* A. Agassiz and H. L. Clark, 1907 (the systematic status of this species is discussed by Swan, 1953, Evolution **7**: 269–273), whose tests varied from 0.5 to 8 cms in diameter.

The procedure for examination of the hosts was essentially that described by Beers (1948, Biol. Bull. **94**: 99–112). The gut was cut into 3 regions (oesophagus, intestine, and rectum) each of which was examined separately. The incidence of infection was almost universal as only 1 (test diameter 1.2 cm) out of 17 individuals examined was free from ciliates. No urchin harbored ciliates in any system except the digestive tract. Regional distribution of species within the intestine agreed with the findings of Beers.

All ciliate material was fixed and prepared by means of the Chatton-Lwoff silver impregnation technique as described by Corliss (1953, Stain Tech. **28**: 97–100). In this preliminary survey entire gut populations were not fixed, but permanent preparations were made of each recognizably different ciliate species. The French silver technique is ideally suited for marine symbiotic and free-living ciliates as the high chloride ion content of the material does not interfere with the impregnation process. The majority of specimens revealed clearly defined nuclei, thus eliminating the necessity of using separate specific nuclear staining procedures.

The following 7 species of holotrich ciliates were identified from the urchins. They represent the first record of the ciliate fauna of *S. echinoides*. *Anophrys elongata* Biggar and Wenrich, 1932; the *"Colpidium echini"* of Russo, 1914 (obviously not a *Colpidium*); *Cryptochilum echini* Maupas, 1883; *Cyclidium stercoris* Powers, 1935; *Entodiscus borealis* (Hentschel, 1924) Madsen, 1931; *Entorhipidium tenue* Lynch, 1929; *Madsenia indomita* (Madsen, 1931) Kahl, 1934.

The following additional forms could not be associated with any previously described species. Proper descriptions are reserved for fuller publication elsewhere. *Plagiopyliella* sp. (intermediate between *P. vorax* Strelkov, 1959, and *P. pacifica* Poljansky, 1951); and a new genus and species of hymenostome ciliate, superficially resembling *Entodiscus borealis*, but differing significantly in nuclear configuration, topography of the buccal apparatus, and number of ciliary meridians.

It is noteworthy to record that species belonging to several orders (Heterotrichida, Peritrichida, and Hypotrichida), previously reported from congeneric hosts of other waters, were not found to be represented in the entocommensal fauna of *S. echinoides*. The lack of any member of the order Gymnostomatida is consistent with the results of all prior investigations of this unique symbiotic economy.

This investigation was made possible by National Science Foundation Training Grant in Zoology, No. G 7045, administered by the faculty of the Department of Zoology, University of Washington, Seattle 5, Washington.—JACQUES BERGER, *Department of Zoology, University of Illinois, Urbana, Illinois.*

EFFECT OF COBALT 60 AND X-RAY ON INFECTIVITY OF CYSTICERCOIDS OF *HYMENOLEPIS DIMINUTA**

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The present experimental work was undertaken as part of an inquiry into the effect of ionizing radiation on certain helminth parasites, and represents a beginning study on the effects of radiation on tapeworms. The organism studied was the rat tapeworm, *Hymenolepis diminuta,* since experimental material was readily available. It was our hope to obtain data as a background for testing the effects of irradiation on the persistence of the parasite in the host following exposure to radiation and upon the injury to the reproductive potential of the parasite. The technique used and the information obtained may be of value in their application to the study of human and other tapeworms which have an analogous life cycle.

MATERIALS AND METHODS

Cysticercoids of *H. diminuta* were reared in the flour beetle, *Tribolium confusum.* Adult beetles were placed in each of several wide-mouthed amber-colored jars, containing about one-half inch of dry, heat-sterilized flour or bran or both. A few drops of brewer's yeast were added to each jar and the jars were then placed in an oven at 30° C. Each week a small piece of apple was added as a moistening agent. Under these conditions the beetles developed from eggs to adults in from 4 to 5 weeks. Adult beetles were screened periodically from the cultures as needed. About 40 or 50 beetles were placed in a 1-inch stender dish and kept without food for 1 week before infecting them with the tapeworm eggs.

Infection of Intermediate Host. Gravid proglottides of *H. diminuta* were teased apart in a drop of physiological saline solution on a glass slide. A small piece of filter paper about 1 inch in diameter was placed in a stender dish. Fragments of proglottides containing eggs were placed on the filter paper to which a small quantity of sugared water had been added. The hungry beetles soon ate the sugar, thus ingesting some of the infective eggs. A piece of moist filter paper was placed on the inner upper surface of the lid of the stender dish to prevent desiccation of the eggs. The beetles were permitted to feed on the eggs from 5 to 10 days. At the end of that time, flour or bran was added to the dish which remained undisturbed for at least 15 days. In about 2 weeks after they began to feed on the eggs, the beetles developed cysticercoids which were infective for rats.

Recovery of Cysticercoids and Infection of Definitive Host. The cysticercoids were dissected from the beetles in saline. Most of the cysts lay free within the hemocoele or were lightly attached to the gut or Malpighian tubules. The cysticercoids were then exposed in saline to various doses of cobalt 60 (one-kilocurie source) and of x-radiation having different energies (245 Kv, 120 Kv and 80 Kv). The cysticercoids, contained in about 3 ml saline solution in a lusteroid tube, were irradiated with cobalt 60 at a dose rate of 633.3 roentgen equivalent physical (rep) per minute; with 245 Kv x-radiation, filtration consisted of 0.5 mm copper and 1 mm aluminum, the distance from tube to cysticercoids was 23 cm and the dose rate was 312 roentgens (r) per minute; with 120 Kv x-radiation, 0.25 mm copper and 1 mm aluminum were used as filters; and for the 80 Kv x-radiation, no filters were used.

Fifteen irradiated cysticercoids were fed by pipette to white rats weighing 175 to 200 g. The rats were killed 12 or 21 days later and the intestines examined for presence and number of

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adult worms. Controls for each experiment consisted of 2 or 3 rats fed no cysticercoids and of 1 to 6 rats (usually 5), each fed non-irradiated cysticercoids.

EXPERIMENTS AND RESULTS

Part I

Effect of cobalt 60. Various doses of cobalt 60 ranging from 5,000 to 30,000 rep were applied to cysticercoids. Table I indicates that a dose of 18,000 rep cobalt 60 rendered the cysticercoids non-infective.

TABLE I. *Number of tapeworms recovered from intestine of rats fed 15 cysticercoids exposed to cobalt 60.*

Experiment No.	No. of rats	Dose of cobalt 60	Day of infection rats were killed	Av. No. tapeworms recovered
1	2	0	12	13.5
	2	18,000		0
2	1	0	12	13
	5	18,000		0
	4	30,000		0
3	5	0	12	11
	5	5,000		12.8
4	5	0	12	14
	5	10,000		12.2
5	5	0	12	5.3
	5	12,000		6.8
6	5	0	12	4.2
	5	15,000		0
7	5	0	12	5
	5	18,000		0
8	6	0	21	13.3
	3	6,000		13
	4	8,000		11.5
	3	10,000		10.3
	4	12,000		5.2
	4	15,000		3.5
	4	18,000		0

A stunted tapeworm that was recovered from a rat 21 days after it was fed cysticercoids that had been irradiated with 15,000 rep cobalt 60 is shown in figure 1. In figure 3 no eggs are present in the proglottides of the stunted worm.

Part II

Effect of x-rays. Cysticercoids were irradiated with x-rays and fed to rats to determine (1) if such cysticercoids could develop within the intestine, and (2) if eggs taken from gravid proglottides of such previously irradiated cysticercoids could develop in the intermediate host, *Tribolium*.

1. Cysticercoids were exposed to 245 Kv, 120 Kv and 80 Kv x-ray. Doses of 8,000 r or more caused reduction in size in some worms that developed. A dose of 15,000 r of 245 Kv or of 120 Kv rendered the cysts non-infective, while this dose of 80 Kv x-ray did not entirely prevent infection (table II).

2. In order to test the infectivity of eggs in tapeworms developing from previously irradiated cysticercoids, an experiment was carried out in which groups of cysts were irradiated with 3 x-ray energies: 245 Kv, at doses of 6,000 r, 8,000 r, 10,000 r, 12,000 r and 15,000 r; 120 Kv, at doses of 8,000 r, 10,000 r, 12,000 r and 15,000 r; and 80 Kv, at doses of 6,000, 8,000, 10,000, 12,000 and 15,000 r. Fifteen cysticercoids exposed to a given dose of x-ray were fed to each of 4 rats. The rats, including controls fed non-irradiated cysticercoids, were killed 28 to 34 days later. No worms were recovered from rats fed cysticercoids exposed to 15,000 r of each of these 3 forms of energy and to 12,000 r 80 Kv x-ray, but tapeworms were recovered from all other rats.

Portions of mature egg-containing proglottides from the tapeworms of these rats were fed to cysticercoid-free control beetles. The beetles were dissected 3 weeks later and cysticercoids were again recovered in all but one instance, demonstrating that the proglottides were usually not sexually sterile, even though some worms were stunted and had malformed proglottides.

When 120 Kv x-rays were used, the tapeworms which developed from cysticercoids that received 12,000 r 120 Kv were sterile except in one instance. In other tapeworms that were examined, the proglottides contained no eggs when viewed microscopically; no eggs were found on flotation of fecal samples of the rat at 21

TABLE II. *Number of tapeworms recovered from intestines of rats 12 days after feeding each rat 15 cysticercoids exposed to various doses of x-ray.*

No. of rats	Dose of 245 Kv	Av. No. tapeworms	No. of rats	Dose of 120 Kv	Av. No. tapeworms	No. of rats	Dose of 80 Kv	Av. No. tapeworms
	r			r			r	
5	0	11.4						
5	4,000	11.2						
5	0	11	4	0	11.2	4	0	12.2
5	6,000	9.8	4	6,000	8.7	4	6,000	10.5
2	0	15						
4	6,000	6.5						
4	8,000	7.7						
4	10,000	3	4	10,000	6.7	4	10,000	6
4	0	13	2	0	14	2	0	10.5
4	8,000	3	4	8,000	8.2	4	8,000	11.7
4	10,000	1.2	4	10,000	3.2	4	10,000	7.2
4	12,000	0.5	4	12,000	0.2	4	12,000	0.7
4	15,000	0	4	15,000	0	4	15,000	0.7

days post-infection, and no cysticercoids were produced in beetles fed macerated portions of the proglottides. No worms were recovered from rats fed cysts exposed to 15,000 r 120 Kv x-ray.

With 80 Kv x-ray, cysticercoids were recovered from beetles fed mature proglottides of worms from rats fed cysticercoids exposed to 10,000 r. No worms were recovered from rats fed cysticercoids exposed to 12,000 or 15,000 r 80 Kv x-ray.

Part III

Effect of X-rays on Cysticercoids by Total Exposure of Intermediate Host, Tribolium. One hundred beetles, given eggs from gravid proglottides 15 days previously, were irradiated with 18,000 rep cobalt 60 in the same manner as the cysticercoids; immediately after radiation, cysticercoids were removed from the beetles and 15 of the cysts were fed to each of 8 rats. When the rats were killed, 25 worms, all stunted, were recovered from the rats (average, 3.1 worms per rat) while 46 worms were recovered from 4 control animals fed non-irradiated cysts (average, 11.5 worms per rat). This experiment suggested that some measure of protection against the radiation is afforded to the cysticercoids by the body of the beetle. Since the body of the beetle provides negligible absorption of the radiation, the protection may be chemical or physiologic in nature.

DISCUSSION

A dose of 18,000 rep cobalt 60 prevented maturation of all cysticercoids to adult tapeworms. In one instance a dose of 15,000 rep applied to cysticercoids produced sexual sterilization of the tapeworms that did develop.

Fewer tapeworms developed after radiation of cysts by x-ray than after comparable doses of cobalt 60. In our previous work on radiation of *Trichinella spiralis* (1957), we observed that the dose of x-ray required to prevent maturation or reproduction was lower than the dose of cobalt 60. In most cases, reduction in number of worms was evident after 6,000 r, 8,000 r and 10,000 r; after 12,000 r only a few tapeworms developed. Doses of 8,000 r or more caused reduction in the size of the worms that developed.

In comparing the results obtained by different energies of x-ray, 245 Kv and 120 Kv x-radiation seemed to be more damaging than 80 Kv in doses ranging from 8,000 to 15,000 r. With the former energies, a dose of 15,000 r was effective in preventing development of any tapeworms.

Alicata (1957) has pointed out that the cysticercoid (as of *H. diminuta*) is a small, compact body whereas a cysticercus (such as that of *T. saginata*) is considerably larger, and that the effective dosage of ionizing radiation in the two different cystic forms may not be the same. One of the objectives of our study was to gain information on range of doses of irradiation required and on techniques.

SUMMARY

Tests were conducted to determine the amount of cobalt 60 or x-ray that was necessary to apply to cysticercoids of *Hymenolepis diminuta*, the rat tapeworm, in order to render them non-infective. Cysticercoids were obtained by feeding flour beetles (*Tribolium confusum*) with infective eggs from the tapeworm; the cysts recovered from the beetles were irradiated, and counted numbers were fed to white rats to determine their ability to produce tapeworms. A dose of 12,000 roentgens equivalent physical (rep) cobalt 60 or 12,000 roentgens (r) x-ray prevented most cysticercoids from developing to tapeworms, and in some cases the few tapeworms that developed were stunted or sexually sterile. Doses of 15,000 rep cobalt 60 or 15,000 r x-ray generally prevented cysticercoids from developing into adult tapeworms.

REFERENCES

ALICATA, J. E. College of Agriculture, University of Hawaii. 1957 Personal communication to the authors.

GOULD, S. E., GOMBERG, H. J., VILLELLA, J. B. AND HERTZ, C. S. 1957 Studies on *Trichinella spiralis*. VI. Effects of cobalt 60 and x-ray on morphology and reproduction. Am. J. Path. **33**: 79–105.

EXPLANATION OF PLATE I.

FIGURE 1. Stunted *H. diminuta* (entire worm), recovered from rat 21 days after it was fed cysticercoids that had been irradiated with 15,000 rep cobalt 60. Natural size.

FIGURE 2. Control *H. diminuta*, recovered from rat 21 days after it was fed non-irradiated cysticercoids. Natural size.

FIGURE 3. Lateral portion of several proglottides of *H. diminuta* recovered from rat 21 days after it was fed cysticercoid exposed to 15,000 rep cobalt 60. No eggs were present. 84×.

FIGURE 4. Lateral portion of several proglottides of *H. diminuta* recovered from rat 21 days after it was fed non-irradiated cysticercoids. Note numerous eggs. 84×.

PLATE I

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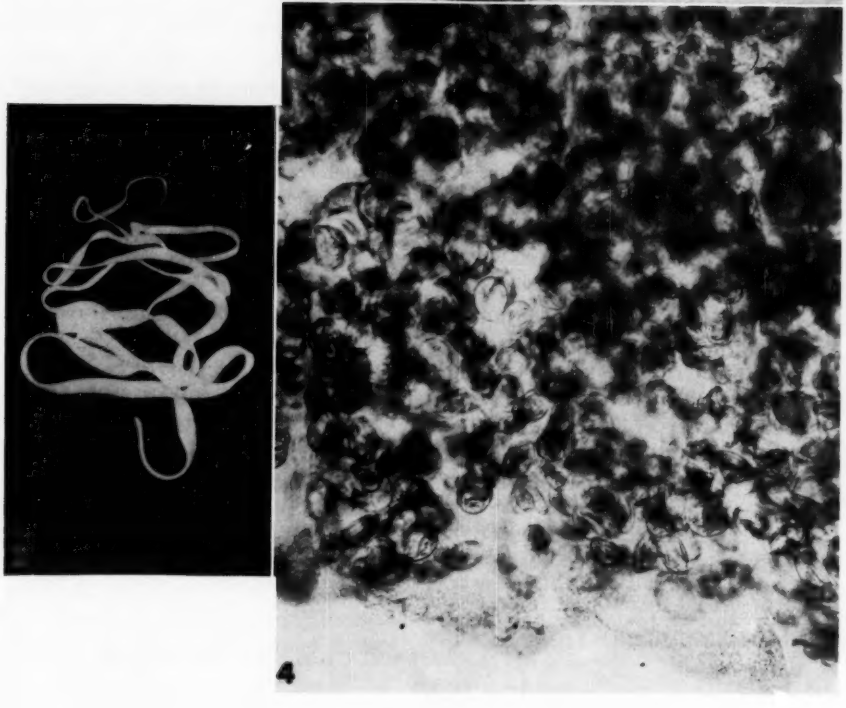

4

GROWTH AND HATCHING OF TAENIID EGGS*

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In our work on the effects of radiation upon *Hydatigera taeniaeformis* Batsch, 1786, we have had difficulty obtaining heavy infections, because of apparent variability among the eggs. In an approach toward control of this variability, we tried to determine the characteristics of a mature egg, as to both its development in the gravid proglottid and its hatchability in artificial media.

The taeniid egg has been described by a number of workers. Wardle and McLeod (1952) described the egg of *Hydatigera taeniaeformis,* the cat tapeworm, and Silverman (1954) described the egg of *Taeniarhynchus saginatus* (= *Taenia saginata*) Goeze, 1782, the beef tapeworm of man, and of *Taenia pisiformis,* Bloch, 1780, the dog tapeworm. Ogren (1953, 1957) outlined the early embryology and development of the embryophore in taeniid cestodes, and gave the taeniid ontogeny a place in his proposed system of embryological patterns. Lee et al (1958) added a note on the origin of the embryophore by secretion. Other workers have described the oncospheres and membranes of members of this important group of tapeworms.

In *Hydatigera,* the developed oncosphere is contained within 3 membranes, an outer "chorionic membrane" enclosing yolk material, a striated embryophore consisting of hexagonal prisms arranged radially and cemented together, and an oncospheral membrane closely investing the 6-hooked larva, or oncosphere. During development, the embryophore probably arises by secretion from a layer of polyploid cells (actually a detached epidermis derived from the embryo during its early cleavage); as the egg matures, the embryophore thickens. The mature egg, liberated by rupture of the proglottid in the host's intestine, loses its outer (chorionic) membrane, and when recovered by concentration from cat feces possesses the characteristic striated appearance of all taeniid embryos.

We undertook the present study in order to determine whether or not the eggs of *Hydatigera* pursue a regular course of development to maturity within the proglottid. As we and others have noted, however, morphological maturity is no guarantee of hatchability in taeniid eggs. Therefore we undertook further studies on the hatching of eggs in artificial media.

Silverman (1954) artificially hatched and activated eggs of *Taeniarhynchus saginatus* with consistently good results, reaching 78 percent hatching in some samples. Whatever differences in percent hatched and activated embryos he obtained were attributed to differences in the percent of mature eggs in the samples used. Our hatching experiments had an unforeseen result, different from Silverman's, which we think has evolutionary implications. We found variation in hatching accompanying apparent uniformity in stages of development.

MATERIALS AND METHODS

Eggs for growth studies and for hatching experiments were obtained from tapeworms from stray cats collected in the city of Knoxville, and from cats experimentally infected at Emory University and the University of Tennessee.

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Measurements of growth were made on 569 eggs of 2 worms from different hosts: 12 proglottids from each worm were used. All eggs to be studied were removed to saline under vaseline-sealed coverslips. An eyepiece micrometer and oil immersion objective were used, with a mechanical stage for random sampling. Length and width of egg, diameter of oncosphere, and thickness of embryophore were recorded. Averages and standard errors were computed and regression lines were plotted corresponding to changes in dimensions from segment to segment. A modified "t" test was used to determine whether or not the regressions were significant (Dixon and Massey, 1957). In the planning and execution of the statistical aspects of our work, the aid of Dr. J. I. Townsend of the University of Tennessee, Department of Zoology and Entomology, is gratefully acknowledged.

In hatching experiments Wantland's (1953) medium, modified by adjusting to pH 7.2 with sodium bicarbonate, was used. Proglottids refrigerated at 10 C for various periods (days to months) in saline were the source of all eggs. After their removal from proglottids, eggs were stored for 3 days under the following conditions: tap water at room temperature; tap water at 10 C; mammalian (0.85 percent NaCl) saline at room temperature; mammalian saline at 10 C. About 3800 eggs were used in these experiments. Other eggs were stored in their proglottids in saline, and were subjected to the hatching solution immediately after being freed. About 2300 eggs, from 17 different proglottids, were used in these experiments. Homogeneity of results of the various replicated tests was established by the chi-square method. Further experiments compared hatchability of newly removed eggs with that of eggs from an adjacent posterior proglottid after 3 days free storage. Eggs of *Taenia pisiformis* were subjected to the same experimental treatment, free stored eggs being compared with freshly removed eggs, in hatching solutions.

RESULTS

The study of development revealed a constant change in certain dimensions of the egg as the proglottid aged. The length of the fully developed egg ranged from 25 to 31 microns. Average length of eggs from the last 6 proglottids of 2 worms was 28.6 microns and 28.2 microns, respectively; the widths were 27.3 and 26.9 microns. The embryophore was 2.7 microns thick. Table I shows average dimen-

TABLE I. *Mean measurements in microns with S.E. of the eggs from one strobila of* H. taeniaeformis.

Source of eggs	No. of eggs measured	Embryophore		Oncosphere		Mean thickness of embryophore
		Length	Width	Length	Width	
A 1–2	30	28.40 ± .21	27.07 ± .20	21.00 ± .25	18.93 ± .20	2.87 ± .07
A 3–4	47	28.70 ± .26	27.30 ± .28	20.00 ± .16	18.57 ± .17	2.96 ± .03
A 5–6	50	28.62 ± .18	27.48 ± .14	21.34 ± .21	19.54 ± .18	2.96 ± .04
A 7–8	50	27.56 ± .14	25.68 ± .20	20.36 ± .20	18.48 ± .19	2.50 ± .08
A 9–10	49	26.84 ± .15	24.41 ± .13	20.00 ± .25	17.86 ± .27	1.50 ± .07
A 11–12	43	26.63 ± .17	23.56 ± .20	18.84 ± .27	16.28 ± .24	1.42 ± .08

sions (with standard error) of the eggs and oncospheres of each proglottid from one worm. Figure 1 illustrates the increase in measured dimensions with proximity of proglottids to the posterior end of the worm. There is steady growth in several dimensions up to the 6th proglottid from the end, while in the last 6 proglottids there is no change. Confidence intervals (95 percent) were calculated for regression coefficients of those data, and the significance of the changes was thereby confirmed. The eggs of *Hydatigera,* therefore become morphologically "mature" before the proglottid detaches; in the ripe segment, eggs are morphologically uniform.

The experiments comparing hatchability of eggs stored under various conditions revealed no differences in hatching among the samples of freed eggs stored for 3

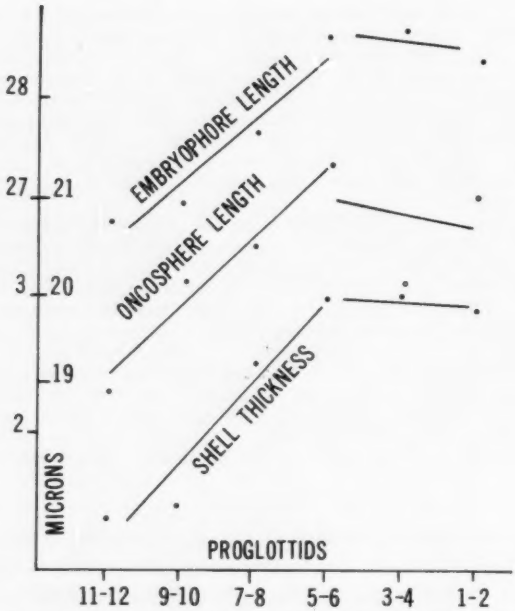

FIGURE 1. Changes in length of egg or embryophore, length of oncosphere, and thickness of shell throughout the terminal 12 proglottids of a strobila; last two proglottids designated by the numbers 1–2.

days or more. In each group about 83 percent of the eggs hatched within 3 hours. Eggs stored for one month in saline at 10 C gave similar results. Hatching occurred gradually, about 5 percent of the eggs having hatched after 1 hour, 15 percent after 1.5 hours, 50 percent after 2 hours, and 83 percent after 3 hours. The rate of hatching was logarithmic, being highest between 2 and 3 hours after exposure to the hatching medium. The above hatchability data contrast with data obtained from eggs placed in the hatching medium immediately after removal from proglottids. Such eggs hatched more promptly than stored free eggs, with about 80 percent having hatched within the first half hour. Eggs from adjoining proglottids when compared in the above manner confirmed these results, those stored in their proglottid hatching more quickly than those stored for the same time free in saline. Eggs of *Taenia pisiformis,* however, hatched in Silverman's (1954) solution with equal speed whether tested immediately after being removed from their proglottid or after 3 days free storage.

DISCUSSION

From the above results we conclude that eggs of *Hydatigera* differ from those of *Taeniarhynchus* in at least 2 respects. Unlike those of *Hydatigera,* the latter are in different stages of development within the same gravid proglottid, and these eggs, after storage, usually increase in hatchability.

Silverman (1954) demonstrated that immature eggs of *Taeniarhynchus* resist artificial hatching, and that a given gravid proglottid may contain from 9 to 78 percent hatchable eggs. Later, Silverman (1955) showed that eggs of *Taeniarhynchus* disseminated in sewage were resistant in various degrees to hatching; some

passed unharmed through avian adventitious hosts, while others were digested and destroyed. Also, he found that storing gravid proglottids in saline for 10 days resulted in an increase in the percentage of hatchable eggs in some (but not all) proglottids tested. The eggs of *Hydatigera,* on the other hand, are nearly all at the same stage of development in the same proglottid, and indeed in the last several proglottids of a strobila. These morphologically similar eggs hatch with relatively uniform speed, however, only immediately after removal from their proglottid. After storage free in saline, their hatchability changes, so that a longer time for hatching is required by a definite percentage of the eggs.

We think the 2 differences mentioned are related to the evolutionary success of each worm which depends in large part on dispersal. Although this fact is so obvious as to need no discussion here, in view of the varied dispersal mechanisms shown in parasite life cycles in general, the usefulness of this principle in understanding the results of Silverman's and our experiments should be mentioned. The apparent paradox, 2 similar species with similar life cycles, differing however in important details of egg development and hatchability, is resolved by considering the relationship to dispersal of both development and hatchability. These variables affect dispersal in time. Variable rates of development as in *Taeniarhynchus* add time (or opportunity for infection of new hosts) to the life cycle, some eggs being ready to hatch when "laid," others only after some time has passed. The latter may be carried far, in time and space, as Silverman (1955) has shown, to distant opportunities. Variable hatchability of morphologically uniform eggs, as in *Hydatigera,* serves the same function. Freed, as they normally are, in the host intestine, these eggs are already partially dispersed when they leave the host. Spread further, by rain or coprophagous animals, they may eventually be ingested by a suitable host. If, then, these eggs hatch after various intervals, some will be carried still farther, and pass unhatched through such hosts to be acquired by others. It is noteworthy in the Knoxville area, at least, that only seldom are more than 2 or 3 larval cysts of *Hydatigera* discovered in a single naturally infected host. Dispersal mechanisms account in part for this sparse distribution. It appears that in both species dispersal in time is effectively accomplished, in *Taeniarhynchus* by varied development and in *Hydatigera* by varied hatchability. It remains to consider what inherent or other factors aid these evolutionarily valuable results.

In *Taeniarhynchus,* the chief factor is probably slow, or perhaps discontinuous, production of fertilized ova, not synchronized with growth or position of the gravid proglottid. This mechanism is seen as an inherent part of the worm's reproductive behavior. Its origin by mutation or recombination and its selection because of its survival value can be assumed. In *Hydatigera,* however, the mechanism is unknown. Some factor must be present in the gravid proglottid that maintains physiological uniformity until the eggs are freed. Thereafter the eggs become more varied. If, in addition to or substitution for such intrinsic factor there is some extrinsic one, some environmental substance with which freed eggs interact, the latter must be present in both cat feces and normal saline. Both kinds of factors, intrinsic and extrinsic, may exist.

Workers wishing to use *Hydatigera* will perhaps be well advised to store terminal and near-terminal proglottids in 0.85 percent salt solution in the refrigerator until time for removing eggs for use in infections experiments. We hope to justify this advice by our own infection experiments in the near future.

SUMMARY

1. Physical growth of eggs in the terminal 12 proglottids was continuous to the sixth proglottid.

2. The last six proglottids contained morphologically mature eggs.

3. Hatching experiments showed uniform hatchability in eggs freshly removed from the terminal six proglottids, even after the proglottids had been stored for various times under varied conditions.

4. Eggs stored after removal from proglottids varied considerably in hatchability.

5. Eggs of *Taeniarhynchus saginatus,* as reported in the literature, differ in both developmental uniformity and experimental hatchability from those of *Hydatigera.*

6. It is suggested that a certain factor in the life cycle of each worm requires peculiar conditions of egg production and storage; dispersal mechanisms essential to survival correspond with such features of the egg as developmental uniformity or variability, and delayed or variable hatching.

LITERATURE CITED

DIXON, W. J. AND MASSEY, F. J. 1957 Introduction to Statistical Analysis. 2d ed. McGraw-Hill, New York.

LEE, H. H-K., JONES, A. W. AND WYANT, K. D. 1958 Development of the taeniid embryophore. Trans. Am. Micr. Soc. In press.

OGREN, R. E. 1953 Concepts of early tapeworm development derived from comparative embryology of onchospheres. Thesis: University of Illinois Graduate School.

——— 1957 Morphology and development of onchospheres of the cestode *Oochoristica symmetrica* Baylis, 1927. J. Parasit. **43**: 505–520.

SILVERMAN, P. H. 1954 Studies on the biology of some tapeworms of the genus *Taenia.* II. The morphology and development of the taeniid hexacanth embryo and its enclosing membranes, with some notes on the state of development and propagation of gravid segments. Ann. Trop. Med. Parasit. **48**: 207–215.

———. 1955 A review of methods of sewage disposal in Great Britain, with special reference to the epizootiology of *Cysticercus bovis.* Ann. Trop. Med. Parasit. **49**: 436–450.

WANTLAND, W. W. 1953 *Cysticercus fasciolaris* in the Syrian hamster. J. Parasit. **39**: 667–668.

WARDLE, R. A. AND MCLEOD, J. A. 1952 The Zoology of Tapeworms. University of Minnesota Press, Minneapolis.

THE NATURAL RESISTANCE OF THE WHITE RAT TO *NEMATOSPIROIDES DUBIUS* AND THE EFFECT OF CORTISONE ON THIS RESISTANCE*

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Nematodes of the genus *Nematospiroides* are rather common parasites of voles over most of North America (Rausch, 1952), and *N. dubius,* in particular, has been reported in many parts of Europe and North America. In Europe the worm has been reported in *Mus musculus* (Schulz, 1926; Fahmy, 1956) and *Apodemus sylvaticus* (Baylis, 1926; Schulz, 1926; Elton et al, 1931; Roman, 1951; Fahmy, 1956). In North America the parasite has been found in *Mus musculus* (Spurlock, 1943; Hall et al, 1955), *Microtus crotorrhinus* (Schad, 1954), *Peromyscus maniculatus* (Ehrenford, 1954), and *Peromyscus leucopus* (Hall et al, 1955).

A survey of the literature indicated that *N. dubius* has been reported only in rodents of the families Cricetidae and Muridae. In the latter family it has been found in the genera *Mus* and *Apodemus.* The observation that it had not been reported in the genus *Rattus* led to this study of the natural resistance of the white rat. A preliminary report of these experiments was presented by Cross and Scott (1956) and more detailed accounts of some experiments are given in a doctoral dissertation (Cross, 1958).

MATERIALS AND METHODS

The strain of *N. dubius* used in these experiments was kindly supplied by Dr. Frank Ehrenford and was originally obtained from a wild *Peromyscus maniculatus gambelii* trapped in California.

The animals used in this study were white rats of the CFW strain and mice of the CF-1 and C3H strains. Feces from infected animals were collected on moist paper towels; cultures made with sawdust were incubated at 23 to 25 C for 6 days. Larvae recovered by use of the standard Baermann apparatus were washed several times and stored at room temperature. Infective larvae were administered by mouth with a small pipette to animals deprived of water 24 hours.

For experiments in which cortisone was employed, some animals received hydrocortisone (supplied by Charles Pfizer and Co., Inc.), others received cortisone acetate. The two types of hormone were equally effective, and in either case the dosage administered was 5 mg per 100 g body weight, except in the instances noted. In most experiments, the cortisone was administered daily beginning on the same day that the infection was introduced. Treatment was discontinued when eggs appeared in the feces, or at least on the 20th day after infection. The hormone was injected subcutaneously into the dorsum of the rat or into the groin of the mouse. In many of the experiments control rats received saline solution (0.9 percent sodium chloride) in the same manner as the hormone and in dosages equalling the volume of cortisone solution administered to the experimental animals.

Beginning with the 8th day of infection, the feces of each animal were routinely examined for *N. dubius* eggs by means of zinc sulfate flotation (specific gravity 1.180).

The animals were killed by ether and the small intestines were immediately removed and placed in 0.85 percent sodium chloride solution. On occasion, portions of the intestine were

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fixed in Bouin's solution, sectioned, and later stained with hematoxylin and eosin. Recovery of the worms involved slitting the intestine longitudinally and laying it open in saline overnight. On the following day the worms were usually free from the intestine and mucus and easily removed. The technique described by McGee et al (1957), which consisted of everting a segment of intestine on an applicator stick and placing this in 1 percent HCl solution, was also employed. The worms were washed in saline, fixed in hot 70 percent ethyl alcohol, and mounted in glychrogel (Scott, 1955). Worm measurements were not made, because no method was available that would allow an accurate measurement of such coiled parasites. To recover encapsulated worms the intestine was cut into 2-inch strips, slit longitudinally, and flattened on the glass plate of a dissecting microscope. The worms could then be seen by transmitted light and removed.

OBSERVATIONS

The Life Cycle of N. dubius *in the Mouse*

The development of the free-living stages of *N. dubius* has been reported by Fahmy (1956) and the complete life cycle of the parasite has been described by Ehrenford (1954). In a moist environment, at optimum temperatures of between 23 and 28 C, the eggs hatch 24 to 26 hours after passing out with the host's feces. The larvae develop into infective 3d-stage forms within 4 to 6 days. Twenty-four hours after ingestion by the host, the larvae are found in the lumen of the small intestine closely associated with the mucosa. After 48 hours the larvae come to lie close to the muscle layers of the intestinal wall, and after 96 hours they are in the 4th stage. The muscle layers of the small intestine are greatly thickened at this time because of hydropic degeneration and leucocytic infiltration (Baker, 1954), and the larvae are grossly visible from the serous surface. The final moult occurs between the 6th and 9th days, i.e., shortly before the worms emerge from the tissues and re-enter the intestinal lumen. Mating and oviposition occur in the intestinal lumen; eggs may be found in the feces as early as the 9th day (Spurlock, 1943; Ehrenford, 1954; Baker, 1954).

In the present study this sequence of events in the life cycle was confirmed and found to be similar in 2 strains of mice. In approximately 100 mice, the prepatent period averaged about 10 days and varied from 9 to 11 days. To determine the period of patency, 12 infections were followed in mice until eggs were no longer detectable in the feces. The period of egg production varied from 4 to 11 months, the average being 8.7 months. Since animals autopsied at the end of egg production did not harbor female worms, it was concluded that cessation of egg production was due to the absence of female worms rather than the inability of the females to produce eggs further.

Autopsies performed at varying times showed that more worms were present in the intestinal lumen of the host between the 10th and 50th days of infection than at any other time. An average of 81 percent of the larvae administered were recovered as adults from 40 animals killed during this period. Between 51 and 100 days of infection an average of 41 percent of the larvae administered were recovered from 5 mice, and in 1 mouse each, killed on the 115th, 264th, and 320th days after infection, 7, 24, and 2 percent, respectively, were recovered.

The Life Cycle in the White Rat

Larvae were administered to white rats and to parallel control mice. Eggs were present in the feces of the mice after 10 days, but were never found in the feces of the rats. Tests to determine whether the larvae were passing directly through the intestine were also negative. Ultimately it was found that in the rat

the larvae penetrate the mucosa and start development as in the mouse. In the rat, however, the inflammatory reaction around the worm was more extensive than in the mouse and involved the formation of a connective tissue capsule. Evidently the worms were not able to break out of this capsule and re-enter the intestinal lumen, as they do in the mouse. The parasites developed a little more slowly in the rat than in the mouse, and at the time when worms in the mouse were laying eggs, some of the worms in the rat were still alive, but they eventually died in the tissue. The tissue reaction and the development of the worms will be described in more detail below.

Since cortisone has been reported to have a suppressive effect on the cellular response (Coker, 1955; Weinstein, 1955), an attempt was made to inhibit the formation of the connective tissue capsules around the parasites by using this hormone. In rats given daily injections of cortisone the larvae developed essentially as they did in mice, re-entering the lumen and depositing eggs only a few days later than in the mouse.

Comparison of Development in Rats and Mice

Simultaneous comparative studies were made on the development of *N. dubius* in mice, in rats treated with saline, and in rats treated with cortisone. Until the 4th day of infection, the parasites appeared to develop similarly in rats and mice, except that at the end of this period the larvae in the rats had not moulted and were still in the 3d stage. On the 5th day of infection, 4th-stage larvae were recovered from a few small nodules visible in the serosal surface of the rat intestine. During the early days of infection the parasites were difficult to recover from the intestinal wall.

On the 6th day of infection worms were readily seen in the intestinal wall of the mouse and the cortisone-treated rat, and in both cases the worms were larger than those found in the saline-treated host.

On the 7th day of infection a few immature adult worms were found in the intestinal lumen of the mouse and the cortisone-treated rat. Conversely, the worms in the saline-treated rat were found only in the gut wall. Although a small number of leucocytes infiltrated around the larvae shortly after their penetration into the intestinal wall of the mouse, no marked tissue reaction was evident until the 7th day of infection. Histological sections of the mouse intestine demontrate developing worms in the gut wall (fig. 1). Leucocytic infiltration was pronounced, but few fibroblasts could be found. As reported by Baker (1954), fibrotic changes are not evident in the mouse until the 15th day of infection, well after the parasite has left the intestinal wall. Similar histological sections of the intestine of the cortisone-treated rat, 7 days after infection, showed the parasites lying in nodules surrounded by relatively few inflammatory cells. In similar histological sections of the intestine of the saline-treated rat the parasite was surrounded by an intense inflammatory and fibroblastic reaction (fig. 2).

With few exceptions, most of the worms on the 8th and 9th days of infection were in the intestinal lumen of the mouse, but in the cortisone-treated rat the majority were still confined to the gut wall. At this time large white connective tissue nodules containing the parasites were observed in the serous surface of the saline-treated rat intestine. Some of the worms recovered from these nodules were dead and partially disintegrated; others were alive but still in the 4th larval

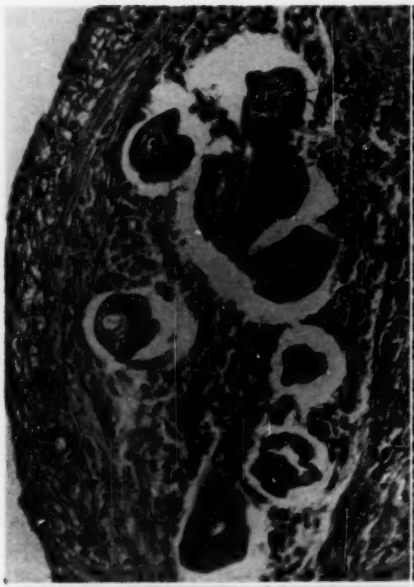

FIGURE 1

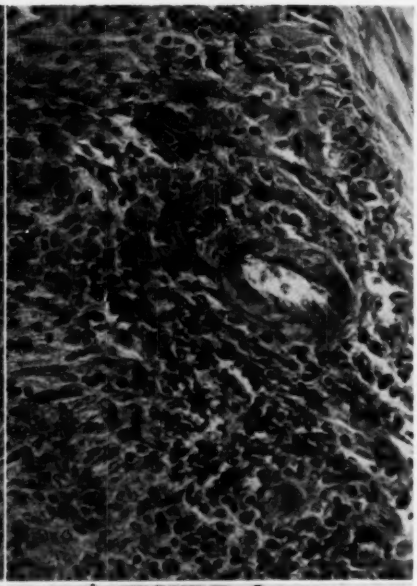

FIGURE 2

FIGURE 1. Section of small intestine of mouse, 7 days after infection, showing leucocytic infiltration which according to Baker (1954) occurs just prior to and following emergence of parasite. × 200.

FIGURE 2. Section of small intestine of rat treated with saline, showing parasite surrounded by an intense inflammatory reaction and fibroblasts 7 days after infection. × 300.

stage. Worms recovered from the mouse were immature adults, but most of those from the cortisone-treated rat were still in the 4th larval stage. Worm recoveries for these 2 days averaged 20, 5, and 66 percent, respectively, for the cortisone-treated rat, saline-treated rat and mouse.

On the 10th day of infection eggs were present in the feces of the mouse but not in the rat. Most of the worms from the intestinal lumen of the mouse were mature adults containing eggs (fig. 3), while those from the intestinal lumen of the cortisone-treated rat were immature adults with no eggs in the uterus (fig. 4). A few living 4th stage larvae were also present in the intestinal wall of the cortisone-treated rat and were surrounded by a few inflammatory cells (fig. 5). Histological sections of the intestine of the saline-treated rat demonstrated the encapsulated parasite surrounded by numerous leucocytes and fibroblasts. Living worms recovered from this rat were much smaller than those recovered from the mouse and from the cortisone-treated rat. Worm recoveries were 52, 2, and 60 percent, respectively, for the cortisone-treated rat, the saline-treated rat and the mouse.

On the 11th and 12th days of infection immature adult worms were found in the intestinal lumen of the rat treated with cortisone. No eggs were present in the uteri of the female worms, but many of the parasites were copulating by the 12th day. The inflammatory nodules in the gut wall of the saline-treated rat were large and contained cellular debris and disintegrated worms. No worms were in

FIGURE 3

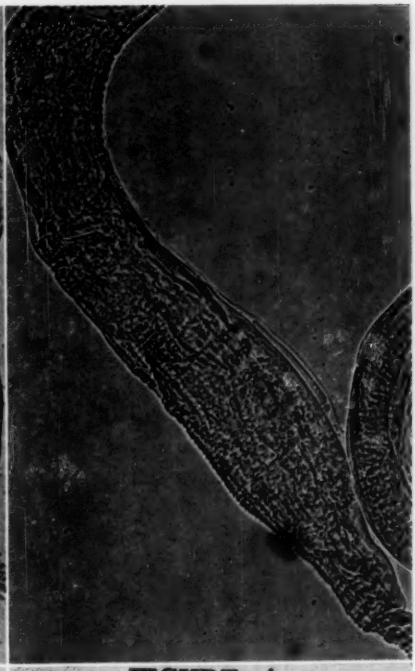

FIGURE 4

FIGURE 3. Posterior portion of female worms recovered from the intestinal lumen of mouse 10 days after infection. Note gravid uterus. × 120.

FIGURE 4. Posterior portion of female worm recovered 10 days after infection from the intestinal lumen of white rat treated with cortisone. Note lack of eggs in uterus. × 120.

the gut wall of the mouse at this time. Worms in the intestinal lumen of the mouse began laying eggs on the 10th day of infection. Worm recoveries were 33, 2, and 68 percent, respectively, for the cortisone-treated rat, the saline-treated rat and the mouse.

On the 13th day of infection eggs were discovered in the feces of the rat treated with cortisone. No eggs were present in the feces of the saline-treated rat, but eggs had been found in the feces of a parallel infected mouse on the 10th day of infection. Although the female worms recovered from the cortisone-treated rat possessed gravid uteri, the number of eggs within the uteri appeared to be less than those in the uteri of the worms recovered from the mouse. Immature adult worms were also recovered from the cortisone-treated rat. The parasites in the saline-treated rat were surrounded by inflammatory cells, cellular debris and a large fibrotic capsule (fig. 6). At this time worm recoveries from the cortisone-treated rat, saline-treated rat, and mouse were 80, 8, and 90 percent, respectively.

The Prepatent and Patent Periods of Infections in Rats

In approximately 75 white rats infected with *N. dubius* and treated with cortisone, eggs appeared in the feces between the 10th and 25th days of infection; the average prepatent period was approximately 12.6 days. To determine the

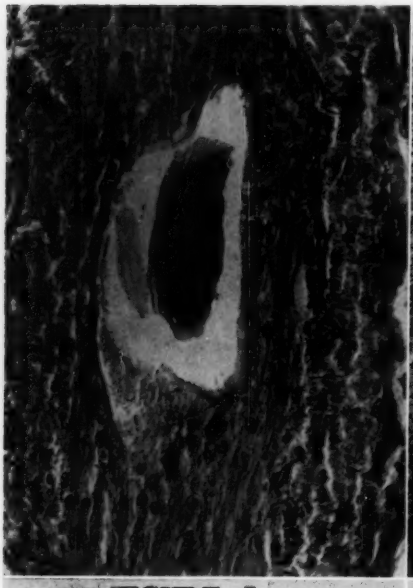

FIGURE 5

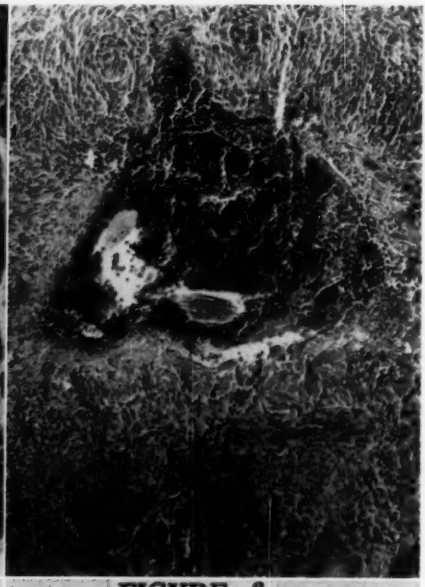

FIGURE 6

FIGURE 5. Section of small intestine of rat treated with cortisone, showing parasite lying in cyst and surrounded by relatively few inflammatory cells 10 days after infection. × 200.

FIGURE 6. Section of small intestine of rat treated with saline showing parasite surrounded by inflammatory cells, cellular debris, and large fibrotic capsule 13 days after infection. × 100.

period of patency in rats, a number of infections were followed until eggs no longer appeared in the feces. The duration of egg production varied from 1 to 4 months; average, 2.4 months. The data from this study, similar to data from mouse studies, indicated that there is little correlation between the number of larvae introduced into the host and the length of the patent period.

As a result of autopsies performed at varying times of infection, it was evident that more worms were present in the intestinal lumen of the rat between the 10th and 40th days of infection than at any other time. During this period an average of 55 percent of the worms administered were recovered from 33 animals examined. Between the 41st and 60th days of infection no worms were present in all rats autopsied; but in one animal each, autopsied on the 72nd and 124th days after infection, 2 and 5 percent, respectively, of the worms administered were recovered.

Infectivity of Larvae from Rat Infections

Studies were made to determine whether progeny from *N. dubius* infections in the cortisone-treated rat possessed the same degree of infectivity as progeny from mouse infections. Infective larvae recovered from rat feces were introduced into rats and mice. Some rats received cortisone acetate while other rats and the mice received no treatment. Additional rats and mice were infected with larvae recovered from cultures of mouse feces and all of these rats received cortisone acetate. The results of the experiment clearly indicated that larvae recovered from the rat infections possessed the same degree of infectivity as larvae recovered from the

mouse infections. Infections developed in rats only when cortisone was administered.

The Life Cycle in the Mouse Treated with Cortisone

A study was made to determine whether cortisone would have any influence on the development of *N. dubius* in the mouse. Two mice received 100 larvae each and cortisone in doses of 5 mg per 100 g of body weight for 3 days, followed by 2.5 mg daily until the 10th day when eggs appeared in the feces. These mice continued to pass eggs for 7 months; at autopsy no worms were found. Two additional mice were given 200 larvae. One mouse received cortisone in doses of 5 mg per 100 g of body weight daily until it died on the 9th day. At autopsy 76 per cent of the worms were recovered. The second mouse was given a similar dosage of cortisone, but treatment was initiated 4 days prior to infection and continued until eggs appeared in the feces on the 11th day. This mouse died on the 12th day, and 61 percent of the worms were recovered. The death of these animals was attributed to the hormone and not to the worm burden. The results obtained from this study seem to indicate that cortisone had little, if any, effect on the development of *N. dubius* in the mouse.

Pre-infection Treatment of the Rat with Cortisone

In an attempt to eliminate the slight delay of development in rats as compared to mice, a group of rats was given cortisone in the usual manner beginning 4 days prior to infection. A control group was treated similarly beginning on the day of infection. Both groups of rats and a group of untreated mice were infected simultaneously. There was no difference between the 2 groups of rats with respect to the prepatent or patent periods, the former being 1 day longer than in mice and the latter much shorter.

Post-Infection Treatment of Rats with Cortisone

Another series of experiments was designed to determine when cortisone administration was required to suppress encapsulation of the parasite. Ten rats were infected and divided into 5 groups of 2 rats each. Group A rats served as controls and were given cortisone starting on the day of infection. Group B, C, D, and E rats received similar doses of the hormone starting on the 4th, 5th, 6th, and 7th days, respectively. Mice not treated with the hormone served as additional controls.

The results of the experiment showed that post-infection treatment with cortisone is not as effective as similar treatment begun on the day of infection. In rats receiving the post-infection treatment of cortisone the prepatent periods for infections were longer than in the control animals, and fewer worms were recovered from the experimental animals than from the controls. It was also found that cortisone treatment must begin before the 7th day of infection, otherwise the parasites will not be able to escape encapsulation and complete their development in the rat.

DISCUSSION

The natural resistance exhibited by the white rat to infections of *N. dubius* appears to have several aspects: first, the connective tissue reaction which surrounds

the developing worm in the intestinal wall of the rat; second, a lower percentage of worm development in the rat as compared to the mouse; and third, a somewhat extended prepatent period compared to that in the mouse.

The foregoing experiments imply that the primary factor in the natural resistance of the rat to infections of *N. dubius* is the connective tissue reaction which surrounds the worm. It has been demonstrated that this nematode develops in the intestinal wall of the rat as it does in the mouse, but slightly more slowly. In the mouse the worm re-enters the intestinal lumen, but in the rat the parasite is apparently unable to do this because it becomes surrounded by a fibrotic capsule. Lindquist (1950) suggested that a cellular reaction around larvae, leading to encapsulation and death, is one mechanism by which abnormal hosts prevent development of certain nematodes which penetrate the skin. It is shown here that the connective tissue response is also a mechanism of natural resistance in abnormal hosts to at least one nematode which penetrates the intestinal wall.

The cellular reaction and subsequent encapsulation of *N. dubius* in the intestinal wall of the rat during the initial infection appears to be similar in some respects to reactions described by other authors. Taliaferro and Sarles (1939), working with hyperimmunized rats infected with *Nippostrongylus muris,* reported that on reinfection the larvae became encapsulated and precipitates were formed in and around them. It was their conclusion that the immunity of rats against *N. muris* was primarily humoral with secondary cellular cooperation. Larsh and Race (1954), working with hyperimmunized mice infected with *Trichinella spiralis,* came to similar conclusions. These authors found that immunity to reinfection with *T. spiralis* results from the direct effects of a humoral factor, and to indirect effects of cellular reaction associated with the resulting inflammation. In studies of initial infection of *N. muris* in an abnormal host, Lindquist (1950) reported that the cellular reaction and retention of worms in the skin and lungs also resembled the reactions described by Taliaferro and Sarles (1939). In order to determine whether humoral factors were involved he made in vitro studies of uninfected abnormal host serum and *N. muris* larvae and was unable to demonstrate a relationship of antibodies to the retention of larvae. In the present studies there was no indication that humoral factors preceded the encapsulation of the parasite in the gut wall of the rat. Precipitates, such as those reported by the above mentioned authors, were not observed around or within encapsulated *N. dubius* larvae.

It has been shown that the resistance of the rat to *N. dubius* could be overcome by the use of cortisone, which suppressed the connective tissue reaction around the developing larvae and allowed the worms to escape encapsulation. On the other hand, cortisone had no effect on the course of infection in the mouse.

The influence of cortisone on helminthic infections has been studied previously by other investigators. Stoner and Godwin (1953) found that cortisone increased susceptibility of mice to trichina infections. Coker (1955) reported that cortisone suppressed the cellular response in the intestinal wall of mice infected with *T. spiralis,* and that the hormone treatment resulted in a longer persistance of more adult worms in the intestine and the establishment of more larval worms in the musculature. Weinstein (1955) found that cortisone administered to rats during or after immunization to *N. muris* caused a marked reduction in the cellular response in the skin to a challenging infection of infective larvae. Roman (1956) has also shown that adult mice, usually resistant to *Strongyloides ratti,* could become in-

fected if they were treated with cortisone. Olson (1957) and Briggs (1958), in their studies with *Litomosoides carinii* in an abnormal host, have also demonstrated that cortisone treatment resulted in a decrease in the number of parasites encapsulated in the pleural cavity.

The fact that fewer worms are found in the cortisone-treated rat than in the mouse further demonstrates the ability of the rat to resist infections of this parasite. The reasons for the lower percentage of worm development in the rat as compared to the mouse are unknown. It is possible that some larvae are unable to enter the intestinal wall of the rat because of the lack of specific penetrating enzymes; or it may be that the enzymes that are present have little effect on the rat intestinal tissue. On the other hand, it is possible that some worms do not have the ability to emerge from the intestinal wall, following growth, because of inadequate enzymes. Then again fewer worms may develop because of the host response, whereby the weak larvae are overcome by some cellular activity which is not influenced by cortisone.

The third expression of natural resistance in the rat to *N. dubius* infections is the somewhat extended prepatent period. In most instances the parasites required a few more days to reach maturity in the rat than in the mouse. The reasons for this retardation are also unknown. It was first thought that one cause may have been the tissue reaction. Although the cellular response was suppressed sufficiently by cortisone to prevent encapsulation of the parasite, there could possibly still have been enough inflammatory activity present to slow down the development of the worm or its emergence. It was thought that pre-infection treatment with cortisone might eliminate this suspected cellular activity, but when rats were treated 4 days prior to infection no difference was found in the development of the worms in the rats pretreated and in rats beginning treatment on the day of infection. Higher dosage levels of cortisone which might have enhanced the development of the worm were not tried for fear that they would result in a rapid death of the animal.

In addition to these observations on the aspects of a natural resistance, certain other facts warrant further discussion. The average number of eggs per female worm at the peak of egg production was less for worms in the rat than for worms in the mouse (Scott et al, 1959). Furthermore, the length of the patent period was less in the rat than in the mouse. In both hosts it was shown that cessation of egg production resulted primarily from death or loss of the worms. The loss of worms occurred gradually rather than suddenly as in the case of the self-cure phenomenon, and therefore may not be a result of acquired immunity.

These differences between hosts might be a result of natural resistance in the narrow sense, or might result from effects which could be included under the natural resistance in a broader sense. In the latter instance the lower egg production and shorter period of patency in the rat might be attributable to the incompatibility of the worms with such environmental conditions as differences in intestinal length (Cameron, 1937), pH, intestinal flora, intestinal motility, or availability of specific nutrients. Factors of this type rather than the tissue reaction are suggested by Newton et al (1959) as explanations of their observation that *N. dubius* does not infect conventional guinea pigs, but does infect germ-free guinea pigs.

Although the life history of the worm in the rat differs from that in the mouse in some respects, larvae produced by infections in both hosts had similar degrees

of infectivity. Some authors have reported alterations in parasites after passage through a new host (Taylor, 1928; Scott, 1930; Haley, 1958). Roman (1956) was able to adapt a strain of *Strongyloides ratti* to mice by administering cortisone to this host. It was shown that progeny of *N. dubius* in the rat were not altered so as to be able to complete their life cycle in the rat without cortisone treatment. It would be of interest to make repeated passages from rat to rat using cortisone with each passage to determine whether a physiological strain could be obtained which would develop in rats without cortisone treatment.

SUMMARY

Nematospiroides dubius develops normally in the white mouse, but the white rat is naturally resistant to this parasite. Larvae penetrated the intestinal mucosa and started development similarly in both hosts. In the rat an intense inflammatory reaction around the larvae resulted in a connective tissue cyst from which the worms were unable to emerge and re-enter the lumen. At the time when worms in the mouse were in the lumen, some worms were still alive in the rat, but they eventually died without leaving the tissues. Cortisone was administered to rats in daily doses of 5 mg per 100 g of body weight in an attempt to inhibit the inflammatory reaction. The worms in these rats developed essentially as those in the mouse, re-entering the intestinal lumen and ovipositing only a day or two later than in mouse infections. Other phases of resistance, such as longer prepatent period, smaller percentage of worm development, and shorter period of patency, were not influenced by cortisone. Treatment with cortisone started 4 days prior to infection did not affect these additional phases nor further influence the development of the parasite in the rat. Treatment with cortisone beginning 4 to 7 days after infection was less effective than treatment started on the day of infection. Cortisone administered to the mouse had no influence on the life cycle of the parasite in it.

LITERATURE CITED

BAKER, N. F. 1954 Trichostrongylidosis: The mouse as an experimental animal. Proc. Am. Vet. Med. As. (91st Ann. Meet. Seattle, Aug. 23–26), pp. 185–191.

BAYLIS, H. A. 1926 On a trichostrongylid nematode from the wood mouse (*Apodemus sylvaticus*). Ann. and Mag. Nat. Hist. **18:** 455–464.

BRIGGS, N. T. 1958 Serological responses of cotton rats and white rats in relation to the extent of infection with *Litomosoides carinii* (Travassos, 1919) Chandler, 1931. Diss. Abstr. 19(5): 1062 Univ. of Mich.

CAMERON, T. W. M. 1937 Concepts and mechanisms of resistance in helminthic infections. Can. J. Res. **15:** 77–90.

COKER, C. M. 1955 Effects of cortisone on *Trichinella spiralis* infections in non-immunized mice. J. Parasit. **41:** 498–504.

CROSS, J. H. 1958 Natural resistance of the white rat to the mouse parasite *Nematospiroides dubius* Baylis, 1926 (Nematoda: Heligmosomidae). Diss. Abstr. 19(6): 1347–1348, Univ. of Mich.

——— AND SCOTT, J. A. 1956 Natural immunity of the white rat to the mouse parasite *Nematospiroides dubius* Baylis, 1926 (Nematoda: Heligmosomidae). J. Parasit. **42** (supp.): 15–16.

EHRENFORD, F. A. 1954 The life cycle of *Nematospiroides dubius* Baylis (Nematoda: Heligmosomidae). J. Parasit. **40:** 480–481.

ELTON, C., FORD, E. B. AND BAKER, J. R. 1931 The health and parasites of a wild mouse population. With a bacteriological note by A. D. Gardner. Proc. Zool. Soc. London, September, pp. 657–721.

FAHMY, M. A. M. 1956 An investigation on the life cycle of *Nematospiroides dubius* (Nematoda: Heligmosomidae) with special reference to the free-living stages. Ztschr. Parasitenk. **17:** 394–399.

HALEY, A. J. 1958 Host specificity of the rat nematode, *Nippostrongylus muris*. Am. J. Hyg. **67**: 331–349.

HALL, J. E., SONNENBERG, B. AND HODES, J. R. 1955 Some helminth parasites of rodents from localities in Maryland and Kentucky. J. Parasit. **41**: 640–641.

LARSH, J. E. AND RACE, G. J. 1954 A histopathologic study of the anterior small intestine of immunized and nonimmunized mice infected with *Trichinella spiralis*. J. Inf. Dis. **94**: 262–272.

LINDQUIST, W. D. 1950 Some abnormal host relationships of a rat nematode, *Nippostrongylus muris*. Am. J. Hyg. **52**: 22–41.

MC GEE, G., RUSSOMANNO, R. AND SANDGROUND, J. 1957 An expeditious method for counting trichostrongylids and other helminths of the small intestine. J. Parasit. **43**: 315–317.

NEWTON, W. L., WEINSTEIN, P. P., AND JONES, M. F. 1959 A comparison of the development of some rat and mouse helminths in germfree and conventional guinea pigs. Ann. N. Y. Acad. Sci. **78**: 290–306.

OLSON, L. J. 1957 Studies on the resistance of white rats and cotton rats to infection with *Litomosoides carinii* (Travassos, 1919) Chandler, 1931. Diss. Abstr. 17(11) : 2574 Univ. of Mich.

RAUSCH, R. 1952 Studies on the helminth fauna of Alaska. XI. Helminth parasites of microtine rodents: Taxonomic considerations. J. Parasit. **38**: 415–444.

ROMAN, E. 1951 Etude ecologique et morphologique sur les acanthocephales et les nematodes parasites des rats de la region lyonnaise. Mem. Mus. Natl. Hist. Nat. Paris, n.s., ser A, Zool. **2**: 49–270.

———— 1956 Specificite parasitaire de *Strongyloides ratti*, du surmulot, Effets de la cortisone sur l'infestation d'autres rongeurs par ce nematode. Ann. Parasit. **31**: 552–571.

SCHAD, G. A. 1954 Helminth parasites of mice in Northeastern Quebec and the coast of Labrador. Can. J. Zool. **32**: 215–224.

SCHULZ, R. E. 1926 Zur Kenntnis der Helminthenfauna der Nagetiere der Union S.S.R. 1. Subordo. Strongylata: 1. Fam. Trichostrongylidae Leiper, 1912. Trudy Gosudarstv. Inst. Eksper. Vet. **4**: 5–32.

SCOTT, J. A. 1930 Further experiments with physiological strains of dog hookworm, *Ancylostoma caninum*. Am. J. Hyg. **11**: 149–158.

———— 1955 Preparation and use of glychrogel mounting medium. J. Parasit. **41**: 219.

————, CROSS, J. H. AND DAWSON, C. 1959 Egg production of *Nematospiroides dubius* in mice and rats. Texas Rep. Biol. and Med. **17**: 610–617.

SPURLOCK, G. M. 1943 Observations on host-parasite relations between laboratory mice and *Nematospiroides dubius* Baylis. J. Parasit. **29**: 303–311.

STONER, R. D. AND GODWIN, J. T. 1953 The effects of ACTH and cortisone upon susceptibility to trichinosis in mice. Am. J. Path. **29**: 943–950.

TALIAFERRO, W. H. AND SARLES, M. P. 1939 The cellular reactions in the skin, lungs, and intestine of normal and immune rats after infection with *Nippostrongylus muris*. J. Inf. Dis. **64**: 157–192.

TAYLOR, E. L. 1928 *Syngamus trachea* from the starling transferred to the chicken, and some physiological variations observed. Ann. Trop. Med. Parasit. **22**: 307–318.

WEINSTEIN, P. P. 1955 The effect of cortisone on the immune response of the white rat to *Nippostrongylus muris*. Am. J. Trop. Med. Hyg. **4**: 61–74.

RESEARCH NOTE

MAINTENANCE OF A MARINE SNAIL IN THE LABORATORY.

The marine mollusk *Batillaria minima* (Gmelin, 1790) was maintained in the laboratory during studies on schistosome cercariae. Since the problem of maintaining the molluscan hosts has impeded experimental work on marine schistosomes the technique is here described.

During the summer of 1958 Dr. L. R. Penner supplied 12 specimens of *B. minima* naturally infected with *Ornithobilharzia canaliculata* (Rudolphi, 1819) Odhner, 1912, from Piney Point, Florida and 15 uninfected specimens from Placida, Florida.

The 12 infected snails were marked with nail polish and placed with the 15 uninfected ones in an enameled pan containing sea water which was changed at monthly intervals. Sticks of blackboard chalk were added and the water was aerated with compressed air passed through air stones. About twice weekly the snails were removed from the aquarium and placed in aerated sea water in a 4-inch finger bowl for feeding, essentially as suggested for the maintenance of *Nassa obsoleta* by Clement (*in* Costello et al. Methods for Obtaining and Handling Marine Eggs and Embryos. Marine Biological Laboratory, Woods Hole, Mass.). The diet for *B. minima* consisted of bits of frozen shrimp, fresh yeast, baker's yeast, scrapings of blackboard chalk and "Vita-Min" bricks (Kagan, Short and Nez, 1954, J. Parasit. **40**: 424–439) and snail food prepared as described by Lee and Lewert (1956, J. Inf. Dis. **99**: 15–20). These foods were offered simultaneously and in excess of the amounts which could be eaten during a 4- to 8-hour period. The snails were then returned to the larger aquarium after food adhering to their shells had been removed with absorbent paper. *B. minima* was observed to ingest all of these materials except the Lee and Lewert snail food on which they sometimes appeared to browse, possibly ingesting small particles. It is doubtful that *B. minima* required all these dietary constituents; however, additional snails were not readily available, and too many constituents were considered preferable to the risk of too few.

After about a month in the laboratory it was noted that the snails were migrating out of the water on the sides of the aquarium. It was found that this behavior could be greatly curtailed by removing the snails from the aquarium at weekly intervals and allowing them to remain dry at room temperature for a period of about 12 hours.

The schistosome infections persisted in all infected snails for at least 9 months, as was made evident by the emergence of cercariae after the snails had been dried overnight and placed individually in vials of sea water under a lamp. Three infected snails were killed after 11 months and the digestive glands fixed in Carnoy's solution. Aceto-carmine squashes were made of some of this tissue, and mitotic figures were seen in young cercarial embryos. After 1 year in the laboratory, all of the uninfected snails were alive; of the 9 remaining infected specimens, 1 had died and cercarial emergence had ceased in 3 others.

This study was made under the direction of Dr. Robert B. Short. The author is also indebted to Dr. William J. Clench for the identification of the mollusks and Dr. Margaret Y. Menzel for making the cytological observations. During this study the author was a Predoctoral Fellow of the National Science Foundation.—ALVIN WAGNER, *Florida State University.* Present address: *Department of Tropical Public Health, Harvard School of Public Health, Harvard University, Boston, Massachusetts.*

CULTIVATION OF *ENTAMOEBA HISTOLYTICA* WITH PENICILLIN-INHIBITED *BACTEROIDES SYMBIOSUS* CELLS. III. THE EFFECTS OF STREPTOMYCIN*

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We are conducting investigations on the effects of antibiotics upon the growth of *Entamoeba histolytica* in a modified Shaffer-Frye (MS-F) culture medium, a technique employing a fluid medium plus penicillin-inhibited cells of *Bacteroides symbiosus*. Some unexpected results were encountered upon adding streptomycin to these cultures. The multiplication of amoebae was found to be dependent upon streptomycin concentration in a complex manner. This antibiotic is regularly employed to assist in the destruction of unwanted bacterial flora during the isolation of new strains of *E. histolytica* from stools or mixed flora cultures, but when the culture is free from contaminating organisms its use has usually been discontinued. Present results indicate that, in certain instances, it may be desirable to continue the addition of streptomycin to the medium in substrate quantities even after contaminating organisms are removed.

Spingarn and Edelman (1948, 1952) had reported an enhanced growth of *E. histolytica* in the presence of streptomycin, but they concluded that the influence of the antibiotic was due to partial control of the mixed bacterial flora. Since the present results were obtained in a medium containing only one species of penicillin-inhibited bacteria, the complications due to the selective action of streptomycin upon a mixed flora are avoided and the question of its probable importance in amoeba multiplication is again raised.

The current findings provide a possible explanation for conflicting statements in the literature concerning the effect of streptomycin upon *E. histolytica*.

MATERIAL AND METHODS

The MS-F technique for the cultivation of *E. histolytica* in the presence of washed, penicillin-inhibited cells of *Bacteroides symbiosus* has been described by Reeves, Meleney and Frye (1957, 1959). The base medium employed in this work contained 0.005 molar cysteine hydrochloride (0.79 gm per liter) in addition to the trypticase, glucose, thiomalate, phosphate, and salt previously listed. The conditions for the cultivation of *B. symbiosus* (ATCC No. 12829) and the preparation, harvesting, and storage of large lots of washed bacterial cells are described in the earlier report (R. M. and F., 1957).

The amoeba cultures routinely contain 12 ml of the autoclaved base medium, 0.25 ml sterile horse serum, 5000 units of penicillin G potassium, and 0.2 ml of a suspension of washed *B. symbiosus* cells containing 0.16 to 0.21 mg of bacterial nitrogen. The usual inoculum consisted of 10,000 amoeba trophozoites suspended in a small volume of fluid from the preceding culture. Total culture volumes ranged from 13 to 15 ml. They were contained in closed screw cap culture tubes and incubation was continued in a slanting position for 48 or 72 hours at 37° C. The amoeba harvests were counted in a calibrated counting chamber following suspension of the cells in the culture fluid. Streptomycin sulfate or dihydrostreptomycin sulfate (Lilly ampoules 431

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and 493, respectively) was dissolved in sterile saline and introduced into the cultures at the time of the amoeba inoculum.

Amoeba strains. The sources of our DKB, 200, F-22, K-9 and JH strains have been indicated in the preceding reports of this series. The BH and NRS strains were recently isolated into MS-F culture from a biphasic egg-slant culture with a mixed bacterial flora. The isolation of the JS strain is described in this report.

All cultures were routinely examined and plated to disclose the presence of any microorganism other than amoeba and *B. symbiosus.* Aside from the first few transfers in the isolation of the new strains, all the reported results were obtained with cultures found to be free of contaminating organisms.

RESULTS

Comparison of streptomycin with certain other antibiotics. Various antibiotics have been tested against the DKB strain of amoeba in the MS-F system (with penicillin). The effects of some of the more strongly amebastatic substances are illustrated in figure 1, curves 1 to 4. In this figure the generations of amoebae

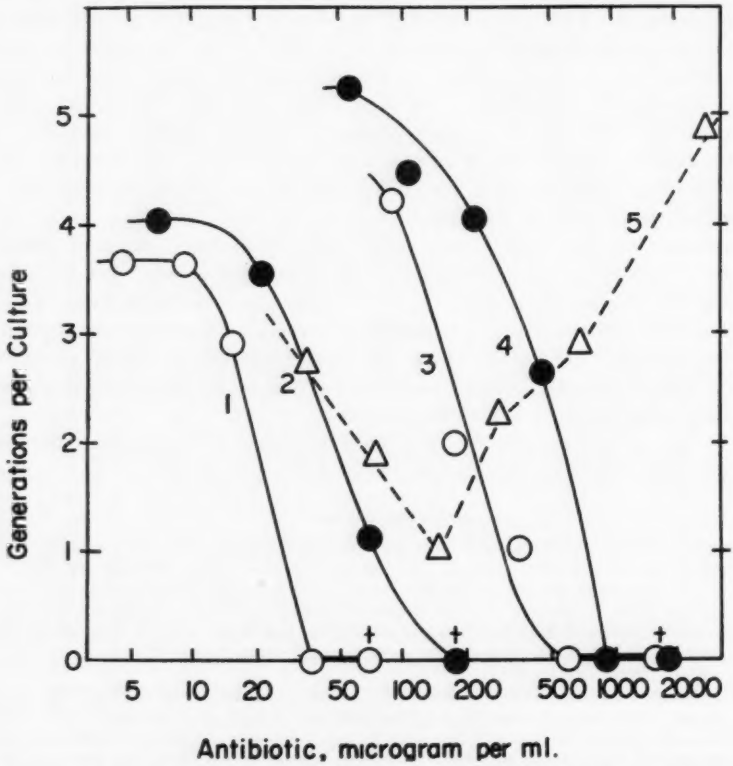

FIGURE 1. Growth of the DKB strain in MS-F culture with antibiotics. 1, tetracycline; 2, oxytetracycline; 3, erythromycin; 4, chloramphenicol; 5, streptomycin. Lowest amebicidal concentration for each of the first 3 antibiotics indicated by cross above the respective experimental point. Standard inoculum, 10,000 cells.

per culture (10,000 cell inoculum) are plotted on the ordinate and the concentrations of the added antibiotics are plotted on a logarithmic scale on the abscissa. These substances, in order of increasing amebastatic effectiveness, and the lowest

observed amebicidal concentration for each (in micrograms per ml) are as follows: chloramphenicol (> 1840), erythromycin (1770), oxytetracycline (178), and tetracycline (69). The peculiar effect of streptomycin with which this report is concerned is illustrated by the broken curve in the figure 1.

The effect of varying concentrations of streptomycin or dihydrostreptomycin on different amoeba strains. Inocula containing 10,000 amoebae were taken from stock MS-F cultures and introduced into tubes containing fresh medium with and without streptomycin. After incubation the amoeba harvests were determined. The results of some typical experiments are illustrated in figure 2. In this figure

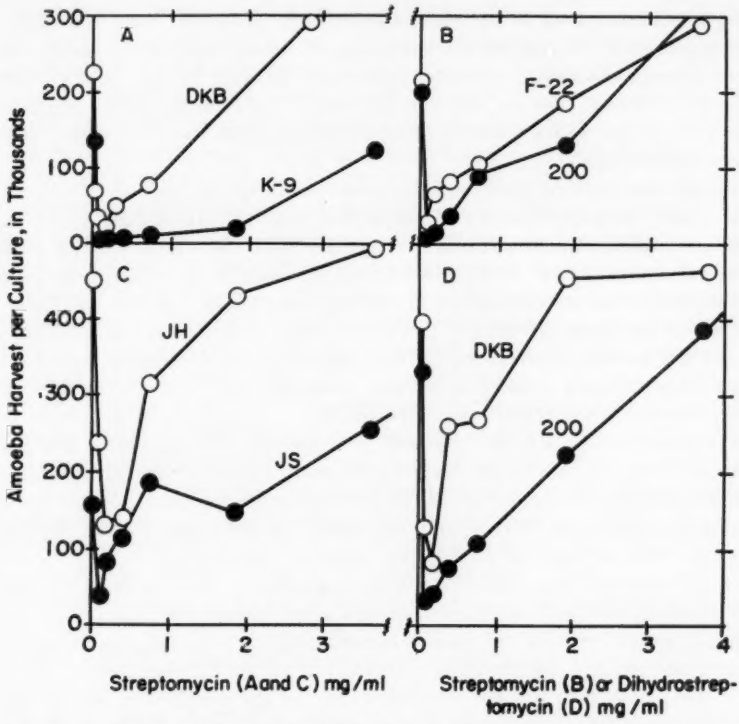

FIGURE 2. Influence of different concentrations of streptomycin or dihydrostreptomycin on multiplication of various strains of *E. histolytica* in MS-F culture. Standard inoculum, 10,000 cells.

the ordinate represents amoeba harvest per culture, the abscissa expresses the streptomycin or dihydrostreptomycin concentration in milligrams per ml. It is apparent by inspection of the figure that low concentrations of these antibiotics (0.05 to 0.5 mg per ml) adversely affect amoeba multiplication, but that at higher concentrations this effect is definitely reversed. Results such as those shown have been obtained repeatedly with all strains of *E. histolytica* examined. It is evident that the effects of these two related antibiotics upon amoeba growth are substantially the same.

In table I are accumulated the results of a number of experiments in which

TABLE I. *Comparison of amoeba harvests in MS-F control cultures with those containing a high concentration of added streptomycin.* (All cultures received a 10,000 cell inoculum.)

Strain of amoeba	No. of expts.	Average amoeba harvest, per culture		Significance of diff. of means
		Without streptomycin	Streptomycin 3.7 mg per ml	
200	5	272,800	563,600	$.02 > P > .01$
DKB	7	278,000	361,900	$P > .05$
K-9	6	297,700	294,200	$P > .05$
F-22	5	199,600	234,600	$P > .05$
JH	4	337,300	323,500	$P > .05$
BH	6	113,500	255,500	$.001 > P$
JS	8	149,500	266,400	$P > .05$

amoeba multiplication in MS-F control cultures is compared with that occurring in the presence of 3.7 mg per ml streptomycin. Inspection of this table reveals that the various strains are somewhat differently affected by this fixed concentration of the antibiotic. In the 200 and BH strains multiplication was significantly increased over that of the control, while in other strains less than significant differences were noted.

Transferring amoeba from streptomycin-containing medium to streptomycin-free medium. It was regularly observed that first transplants did poorly when inocula were taken from a tube rich in streptomycin to the regular culture medium. Simple calculation showed that sufficient antibiotic was being carried over with the inoculum to produce concentrations in the inhibitory range. A culture of the K-9 strain which had been carried for 18.4 generations (4 transfers) in medium containing 4 mg per ml of streptomycin was sedimented by low-speed centrifugation and the amoebae were washed twice with base medium. They were then resuspended, counted, and 10,000 cells were introduced into each of 3 culture tubes. The first contained no streptomycin and produced 309,000 amoebae. The second contained 0.076 mg per ml of streptomycin and produced only 51,000 amoebae. The third contained 4 mg per ml of the antibiotic and produced 274,000 amoebae. This experiment shows that a first transplant on streptomycin-free medium is successful if the inoculum is washed free from the antibiotic.

Experiments omitting penicillin. When penicillin is omitted from the regular MS-F cultures, bacterial overgrowth occurs which causes the cultures to become cloudy and greatly reduces the amoeba harvest. When penicillin was omitted from a culture containing 4 mg per ml of streptomycin, a similar phenomenon occurred. In this case overgrowth was not so pronounced, but the medium became turbid, the bacterial sediment increased, and the amoeba harvests were markedly depressed.

Testing the effect of the sulfate ion. Since both the streptomycin and dihydrostreptomycin were employed as their respective sulfate salts, it was necessary to ascertain whether or not any of the observed findings should be attributed to this anion. Two series of experimental tubes were prepared containing 0, 0.14, 0.35, 0.7, 1.8, and 3.6 mg per ml sodium sulfate. To each tube of one series was added sufficient streptomycin to produce a final concentration of 0.15 mg per ml. No streptomycin was added to the tubes of the other series. All tubes received the standard 10,000-cell inoculum. The harvests from the streptomycin-containing cultures were not influenced by the sulfate concentration, and averaged 45,600 amoebae. The harvests from the streptomycin-free cultures were likewise not influenced by the sulfate concentration, and they averaged 212,500 amoebae. This experiment indicates that neither the amebastatic effect at low concentrations nor

the reversal of this effect at higher concentrations can be attributed to the sulfate anion.

Testing the effect of streptidine. Streptidine sulfate (a sample of which was supplied by Mr. M. McCowen, Eli Lilly and Company) was dissolved directly in the base medium which was then sterilized by filtration rather than by autoclaving. Separate experiments had established the fact that the filtered medium was suitable for amoeba growth. Five experimental tubes were set up to contain final concentrations of 0, 0.43, 0.86, or 1.72 mg per ml streptidine sulfate or 3.7 mg per ml streptomycin, respectively. All were inoculated with 10,000 amoebae of the BH strain taken from a streptomycin-containing culture. The streptomycin carryover was calculated to amount to 3 mg per culture or approximately 0.21 mg per ml final concentration in the new tubes. This concentration is within the range in which growth of the BH strain is strongly suppressed. The amoeba harvests from the cultures containing no streptidine and increasing concentrations of streptidine were as follows: no streptidine, 11,250 amoebae; 0.43 mg per ml, 7,860 amoebae; 0.86 mg per ml, 10,900 amoebae; and 1.72 mg per ml, 11,600 amoebae. From the culture containing added streptomycin the harvest was 169,000 amoebae.

All the tubes with the low streptomycin concentration showed depressed growth, irrespective of streptidine concentration. No significance is attached to the slight variation among these harvests. However, the carryover of a small amount of streptomycin was not harmful when the culture contained a large amount of added streptomycin. This experiment shows that streptidine cannot replace streptomycin in overcoming the amebastatic effect of low concentrations of the latter.

Isolation of the JS strain. A fluid stool specimen containing viable trophozoites was obtained from a patient at a local hospital. One-tenth ml was inoculated into a tube of MS-F medium containing 50,000 units of penicillin (10 times the usual amount) and 100 mg streptomycin. Excellent growth of *E. histolytica* occurred accompanied by a visible yeast contaminant. By the 3d transplant in the same medium, yeast overgrowth appeared imminent. Five mg of solid amphotericin B was introduced into the culture in a finely divided state and the yeast growth declined abruptly. Some of this solid was carried over into the next few transplants; by the 5th transplant the culture was free of detectable contaminating organisms. On the 4th transplant, 2 tubes were set up, 1 with 50 mg streptomycin and 5000 units of penicillin, the other lacking streptomycin. The amoebae in the latter transplant failed to multiply, but those in the former multiplied and the culture was continued from this line. Growth was excellent averaging 274,000 (4.8 generations) amoebae per culture in 17 subsequent transplants in streptomycin-containing medium. After 20 transplants in this medium, a line was transferred to the regular MS-F medium (lacking streptomycin). Ten thousand amoebae were planted but only 6,700 were harvested from the 1st of these cultures. These were concentrated by centrifugation and replanted in a 2d tube from which the harvest was only 1,600 amoebae. These were replanted and the harvest from the 3d transfer was 20,000, a 12.5-fold increase (3.6 generations). On subsequent transfers the harvests per culture with a 10,000 cell inoculum rose to above 100,000 amoebae, and the strain became established in a streptomycin-free medium. The streptomycin carryovers in the first 3 transfers were calculated to be 0.11, 0.013, and 0.0017 mg per ml, respectively, if no antibiotic was destroyed.

Isolation of the NRS strain. An inoculum was taken from a biphasic culture

with a mixed bacterial flora into the MS-F medium containing a high concentration of streptomycin. The isolation proceeded about like that described above for the JS strain, except that the complication due to yeast contamination did not occur. By the 3d transfer the cultures were free from contaminating organisms and the strain is being currently maintained in a medium containing 3.7 mg per ml of streptomycin. After numerous attempts, a line of this strain was established in the control MS-F medium, but growth was usually very poor and this line was ultimately discontinued. Prior to the recognition of the importance of streptomycin in the medium several unsuccessful attempts had been made to isolate this strain into MS-F medium (R. M. and F. 1957).

DISCUSSION

The foregoing results clearly demonstrate the wide differences between the effects of streptomycin (or dihydrostreptomycin) and a number of other antibiotics upon the multiplication of *E. histolytica*. When a function of culture growth is plotted against the concentration of an amebicidal or amebastatic substance in the manner of figure 1, the usual pattern of results resembles that of curves 1 to 4. This pattern features a sharply descending growth curve with increasing concentration of the added substance. The transition from negligible amebastatic properties to complete stasis usually occurs in one order of magnitude of concentration change, as is the case with these curves. The lowest amebicidal concentration is usually somewhat greater than that which is merely amebastatic. In this connection it is noted that the reported results with oxytetracycline and chloramphenicol (and unreported results with emetine) fall within the respective ranges of values obtained by other workers, as reported in the review article by Balamuth and Thompson (1955).

At low streptomycin concentrations, in the range of approximately 0.05 to 0.5 mg per ml, the multiplication of amoebae is strongly depressed. However, this effect is reversed at higher concentrations. At a concentration of 3.7 mg per ml the amebastatic effect is completely reversed and the multiplication of amoeba occurs to an extent equal to or greater than that occurring in the respective streptomycin-free controls. It does not seem plausible to suppose that the direct effect of the antibiotic upon the amoebae is different at low and high concentrations. It is more likely that low concentrations of the antibiotic adversely affect some previously unrecognized metabolic activity in the penicillin-inhibited *B. symbiosus* cells, an activity which provides some product or condition essential to amoeba growth. This supposition is supported by the observation that all strains of amoeba are similarly affected by the same low concentrations of streptomycin. But this concept requires that at higher concentrations the amoebae are able to supply their need directly from the antibiotic. The idea of direct interaction between amobae and antibiotic at higher concentrations is supported by the observed differences among the strains in this concentration range. This rather unusual hypothesis is presently undergoing further investigation.

Experiments have shown that the streptomycin effects cannot be produced by the isolated streptidine moiety of the antibiotic molecule. Nor were they due to the sulfate anion accompanying the antibiotics. There was no indication that streptomycin acted by releasing *B. symbiosus* from penicillin inhibition. The strep-

tomycin-containing cultures remained clear and the bacterial sediment disappeared as the multiplication of amoebae progressed, exactly as occurs in the control cultures. When penicillin was omitted, the streptomycin did not completely suppress the growth of the bacteria, but in these instances the harvests of amoebae declined.

Upon transferring amoebae from a streptomycin-containing medium to one lacking this antibiotic, poor growth was observed in the first few transfers. This suggested that the amoebae might have become streptomycin-requiring and needed several passages to recover the ability to grow without the antibiotic. However, further experimentation showed that the poor growth was undoubtedly due to the carryover of antibiotic along with the inoculum, and that by washing the inoculum free from streptomycin the lag could be eliminated. This finding is of practical importance in the isolation of new strains of *E. histolytica*. In the present work the JS and NRS strains would have been lost at an early stage if a parallel line had not been maintained on streptomycin-containing medium.

Although Spingarn and Edelman (1948) first believed that they had observed enhancement of amoeba growth due to streptomycin (they employed 1 to 10 mg per ml), in their later publication (1952) they somewhat discounted this possibility. They concluded that the favorable effect of the antibiotic was probably due to suppression of unfavorable bacterial flora. Since the present culture methods are quite different from those employed by Spingarn and Edelman it cannot be certain that the former authors did indeed encounter growth enhancement due directly to streptomycin, but this conclusion gains credibility.

Anderson and coworkers (1946) had found relatively dilute solutions of streptomycin to be amebacidal, while Balamuth and Wubold (1946) had observed no harmful effect upon trophozoites at streptomycin concentrations as great as 10,000 units per ml. The latter workers employed the NRS strain which is one now found to respond with increased multiplication to such concentrations of the antibiotic. On the other hand the concentration employed by Anderson et al (1 : 2500 dilution of a preparation containing 350 units per mg.) would place their concentrations in the range presently found to be strongly amebastatic. The current results would appear to substantiate both of these earlier findings, which had hitherto appeared to be mutually contradictory.

SUMMARY

Seven strains of *E. histolytica* in MS-F culture showed strong suppression of multiplication at streptomycin concentrations from 0.05 to 0.5 mg per ml. A similar result was obtained with dihydrostreptomycin in the two strains investigated. At streptomycin concentrations in excess of 2 mg per ml, the amebastatic effect is reversed and the multiplication of amoeba is resumed. With some amoeba strains multiplication was significantly increased above that of the controls by the incorporation of 3.7 mg per ml streptomycin into the medium.

The effects of streptomycin are not due to the sulfate anion which accompanied it, nor are they reproduced by the streptidine moiety of the streptomycin molecule.

The isolation of new strains of amoeba from stools (or mixed flora cultures) into MS-F culture may be facilitated by prolonging the use of streptomycin beyond the period necessary for the control of contaminating bacterial flora.

In transplanting amoeba from streptomycin-rich media to streptomycin-free media, multiplication of amoebae in the first transfer is enhanced if the inoculum is washed free from streptomycin.

A possible explanation for the action of streptomycin on the amoeba cultures is discussed.

References

ANDERSON, H. H., VILLELA, G. G., HANSEN, E. L. AND REED, R. K. 1946 Some physical and biologic properties of subtilin and other antibiotics. Science **103:** 419–420.

BALAMUTH, W. AND THOMPSON, P. E. 1955 Pp. 277–345 *in* Hutner, S. H. and Lwoff, A. Biochemistry and Physiology of Protozoa. Academic Press, Inc., New York.

BALAMUTH, W. AND WUBOLD, M. L. 1946 Action of streptomycin on *Endamoeba histolytica* in vitro. J. Parasit. **32** (supp.) : 10.

REEVES, R. E., MELENEY, H. E. AND FRYE, W. W. 1957 A modified Shaffer-Frye technique for the cultivation of *Entamoeba histolytica* and some observations on its carbohydrate requirements. Am. J. Hyg. **66:** 56–62.

———, ——— AND ——— 1959 The cultivation of *Entamoeba histolytica* with penicillin-inhibited *Bacteroides symbiosus* cells. I. A pyridoxine requirement. Am. J. Hyg. **69:** 25–31.

SPINGARN, C. L. AND EDELMAN, M. H. 1948 The use of streptomycin in the cultivation of *Endameba histolytica* from stools. Am. J. Trop. Med. **28:** 825–829.

——— AND ——— 1952 Further observations on the use of streptomycin and penicillin in the cultivation of *Endamoeba histolytica* from stools. Am. J. Trop. Med. Hyg. **1:** 412–416.

STUDIES ON EXCYSTATION OF COCCIDIAL OOCYSTS*

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There is a wealth of seemingly contradictory findings in the literature concerning the mechanism of excystation, in the gut of the host, of the oocyst of various species of the Genus *Eimeria*. The present study was undertaken in an attempt to clarify the details of this phase of the life cycle by attempting to produce excystation under various experimental conditions.

MATERIALS AND METHODS

The strain of *Eimeria nieschulzi* used was isolated from wild rats taken in the Bronx Zoological Gardens and propagated in albino laboratory rats. Species identification was made by comparing oocysts, prepatent and patent periods, and endogenous stages with those described by Roudabush (1937). Non-pedigreed white rats 2 to 4 months old were utilized as the host in all the experimental work. The hosts were kept in wire mesh cages with a removable wire mesh floor, and were maintained on a diet of commercial "lab chow" supplemented with fresh carrots.

The digestive enzymes used were dry commercial products which were prepared for use in 0.5 percent solution. The pepsin solutions were adjusted to pH 2.0 and the other enzyme solutions were adjusted to pH 7.5 by the addition of dilute HCl or Na_2CO_3. The proteolytic, lipolytic, or amylolytic activity of these solutions was verified.

Host gastric and intestinal juices were obtained by sacrifice of the rats and recovery of the contents of their stomachs or small intestines. These juices were filtered prior to use.

Commercial dry beef bile made up in 10 percent solution, fresh dog bile, and fresh rat bile were used as one type of oxido-reduction system. Leuco methylene blue—methylene blue and ethanol—acetaldehyde were used as another type of redox system.

Oocysts were recovered from host fecal material collected daily in 2.5 percent $K_2Cr_2O_7$. Pellets were crushed in water and sedimented in the centrifuge with the supernatant being discarded. The sediment was washed with distilled water repeatedly until the supernatant was clear; and following discard of the clear supernatant, a flotation fluid was added to the sediment, the two were mixed, and the mixture centrifuged slowly for several minutes. The oocysts in the surface film were removed with a wire loop and placed in distilled water. Both $ZnSO_4$ and sucrose solution were employed as the flotation medium. The recovered oocysts were then washed and placed in 2.5 percent $K_2Cr_2O_7$ solution for sporulation. Following sporulation, the oocysts were washed at least 5 times with distilled water. Washed suspensions of sporulated oocysts were administered to the host either by feeding in milk suspension or by syringe force-feeding in saline.

In all of the in vitro experiments, incubation of the oocyst suspensions was in either Pyrex brand or Pyrex brand and Kimble brand glassware, and was carried on either at room temperature or at 37.5° ± 0.5°C. In all cases, a drop of the sediment was removed from the incubation tube at intervals for microscopic examination for evidence of excystation. In all instances, washed, sporulated oocysts were used.

Oocysts were exposed to the action of host digestive juices by adding approximately 2000 oocysts to a centrifuge tube containing 2 ml of the digestive juice and incubating.

Oocysts were exposed to a change in redox potential by adding approximately 2000 oocysts to a centrifuge tube containing 5 ml of approximately 0.1 per cent HCl; 0.5 ml of the electron donor was then added to the tube and the tube was incubated for 30 minutes. The HCl was then neutralized with 0.1 percent NaOH and the tube incubated further for 4 hours.

Oocysts were exposed to change in pressure by suspending oocysts in distilled water, saline,

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and solutions of digestive enzymes in a closed test tube which could be connected to the air pressure line or the vacuum line. The suspensions were then exposed to either 30 pounds of air pressure or to a vacuum of approximately 28 inches of mercury for 30 minutes. During exposure the tube was shaken vigorously.

RESULTS

In all of the trials listed in table I, and all were repeated at least 6 times, no

TABLE I. *In vitro exposure of washed sporulated oocysts to the action of various agents.*

Agent	Incubation Time	
	Room Temperature	37.5° ± 0.5°C
Pepsin	30 min. to 24 hrs.	30 min. to 24 hrs.
Trypsin	30 min. to 24 hrs.	30 min. to 24 hrs.
Pancreatin	30 min. to 24 hrs.	30 min. to 24 hrs.
Lipase (Pancreatic)	30 min. to 8 hrs.	30 min. to 8 hrs.
10 pct. beef bile	4.5 hrs.	4.5 hrs.
Fresh dog bile	4.5 hrs. total	4.5 hrs. total
Fresh rat bile	4.5 hrs.	4.5 hrs.
Leuco methylene blue-methylene blue	4.5 hrs.	4.5 hrs.
Ethanol-acetaldehyde	4.5 hrs.	4.5 hrs.
30 lbs. air pressure	30 min.	30 min.
28 in. Hg vacuum	30 min.	

evidence of free sporocysts, free sporozoites or disruption of the oocyst wall was found. In addition, washed sporulated oocysts were exposed to a pepsin-trypsin sequence as outlined by Krijgsman (1926) many times without finding evidence of excystation.

The mammalian intestinal mucosa and its associated digestive glands are tissues which carry on vital processes not directly related to digestive enzymes or digestion. To test the hypothesis that excystation of coccidial oocysts may be dependent upon a physiological effect other than that of digestive enzymes, fresh cultures of sporulated oocysts were washed repeatedly (with partial gravitational settling and centrifugation) to obtain a suspension of oocysts as free of bacteria and debris as possible. The oocysts were suspended in sterile saline following the last washing. Rats were then anesthetized with sodium pentobarbitol, given an injection of heparin and, following cannulation of the femoral or jugular vein, the suspension of oocysts was injected directly into the blood stream. The rats were sacrificed after 30- and 60-minute intervals and portions of the lung taken for sectioning. The discovery of empty oocysts in the alveolar blood vessels in sections made from this lung tissue seemed to be evidence that excystation can occur in the blood stream. However, when oocysts were placed on a warm slide with fresh blood, no evidence of excystation could be found during an hour's time.

Since excystation does occur in the blood-vascular system, it was suspected that it might occur also in other tissues. In view of the observations of Van Doorninck and Becker (1957) with *E. necatrix*, that macrophages can transport sporozoites without injury to the parasite, a more extensive set of experiments was planned. A relatively clean suspension of oocysts was prepared as outlined above. Approximately 10,000 oocysts in 0.5 ml of sterile saline were then injected into the thigh musculature of one rat and into the peritoneal cavity of another. In addition, a third rat was fed the same dosage from the suspension used for the other 2 animals. In all 3 animals, oocysts appeared in the feces at the end of 7.5 days. The orally infected animal produced considerably larger numbers of oocysts than did either of the other two animals.

To establish definitely that the infection in the injected animals came from the injected oocysts, the experiment was repeated with some modifications. In these experiments, 2-compartment cages were used, with the 2 compartments separated only by a metal partition. An experimental animal was placed in one compartment, while a non-infected, non-injected control was placed in the second compartment of each cage. In all cases, the controls remained free of infection. In one series of experimental animals (series No. 1), the saline suspension of approximately 10,000 oocysts was injected into the thigh musculature. In a second series of experimental animals (series No. 2), a similar injection was made into the peritoneal cavity. In an attempt to preclude the possibility that an infection might arise as a result of the rat licking the needle wound and thus ingesting any oocysts which might ooze out of the wound before it closed, the wound and the surrounding area for about two millimeters were coated by spraying with Aeroplast (Aeroplast Corporation) following withdrawal of the needle. In this set of experiments, the injected animals began producing oocysts at the end of 7.5 days while the control animals remained free of infection. The oocysts produced by the animals infected by injection were normal in appearance and sporulated in the time usually required by the species. These oocysts, when administered orally or by injection, were infective to other rats, and the prepatent and patent periods of the resulting infection was normal, as were the numbers of oocysts produced. A total of at least 3 animals was included in each series.

The second type of injection experiments was repeated with the exception that 3 rats each of series 1 and series 2 which had recovered from an injection-induced infection were used. Seven and one-half days after injection of oocysts into the thigh musculature or peritoneal cavity, these animals began producing oocysts. Following recovery, series 1 rats were fed an infective dose of sporulated oocysts while the series 2 rats were again given an intraperitoneal injection of the oocyst-saline suspension. In addition, two other rats (series 3), which had previously been infected orally and were now known to be refractory to reinfection by the oral route, were given an intraperitoneal injection of the oocyst-saline suspension. Fecal examinations were made of all of these animals from the seventh to the twelfth days following administration of the infective dose. No oocysts were found which would sporulate although a few (less than 50 for each animal) oocysts which appeared intact and viable were recovered from some of the hosts of all 3 series. However, objects in moderate numbers were recovered from the feces of experimental animals of all 3 series from the 8th to the 12th days which appeared to be incomplete or degenerate oocysts. These objects were of the same size as the oocysts of *E. nieschulzi*. Many of them had a limiting membrane of the same appearance as normal oocysts, while some of them possessed a limiting membrane which was considerably thinner than the oocyst wall. In some of these objects, the wall could be seen to be made up of a series of irregularly shaped flattened plaques. The interior of some of these appeared structureless or empty, while others presented a vacuolated appearance. Some of those which appeared structureless nevertheless appeared to be partially plasmolized in the flotation solution used for recovery.

In an attempt to determine the details of excystation, rats were anesthetized with sodium pentobarbitol, the shank musculature was exposed, and injections of the oocyst-saline suspensions were made into the gastrocnemius and soleus muscles. The animals were sacrificed at 30- and 60-minute intervals, and the muscles taken

for sectioning. No oocysts in the act of excysting were found in sections of these tissues, although free sporozoites as well as intact oocysts were found.

DISCUSSION

Although it is possible that different factors favor excystation in different species of *Eimeria* from different vertebrate hosts, such a possibility appears unlikely. Negative results with in vitro studies must be interpreted with caution; however, this and other studies indicate that proteinases and, in particular, activated trypsin do not represent the single causative agent of excystation for several species of *Eimeria*. Whether enterokinase-activated trypsin is present in the digestive tract of the centipedes and millipeds known to be parasitized by species of *Eimeria* is unknown. Cathepsin is present in skeletal muscle of rats and plasminogen has been isolated from the blood plasma of some vertebrates. Whether trypsin or other proteinases are present in the serous fluid of the peritoneal cavity of rats is uncertain. The experiments with digestive enzymes and fluids and those with injected oocysts seem to indicate that excystation is not directly dependent upon digestive enzymes, at least for hydrolysis of the oocyst wall.

The identification of the outer layer of the oocyst wall by Monné and Hönig (1954) as a quinone-tanned protein which is resistant not only to proteolytic enzymes, but also to the action of keratin solvents and alkalies, appears to discount the role of proteolytic enzymes in excystation. Whether the remainder of the oocyst wall is composed of lipid or polysaccharide is disputed. Cytochemical evidence such as that presented by Pattillo and Becker (1955) for *E. brunetti* and *E. acervulina* indicates that lipid is not present in the oocyst wall and that the non-protein portion of the wall is composed of polysaccharide which is not acid mucopolysaccharide of the hyaluronic or chondroitin sulfuric acid type. Cytochemical analysis of the oocyst wall of *E. nieschulzi* by the author indicates that the wall is composed of protein and polysaccharide.

Monné and Hönig (1954) postulated the presence of a chemical substance (enzyme?) in the protoplasm of coccidian oocysts which is responsible for excystation. This postulate is based upon the observation that stored cultures of oocysts may undergo internal destruction. The present author has observed this phenomenon in both sporulated and non-sporulated oocysts. However, with reasonably fresh suspensions, such changes occur in only a few of the oocysts present, and it has always seemed possible to attribute such destruction to the action of bacteria which had penetrated the oocysts.

The passage of apparently unaltered unsporulated and sporulated oocysts through the digestive tract following an infective feeding suggests that excystation is a vital phenomenon which requires viable sporulated oocysts.

If proteolytic action on the oocyst wall is not a single (but perhaps contributing) cause of excystation, then hydrolysis of the polysaccharide portion of the wall may be postulated as a cause. However, the resistance of sporulated oocysts to the action of amylolytic digestive enzymes in the in vitro studies seems to preclude this possibility. Whether the high energy phosphate system of the host plays any role in excystation is uncertain. It is possible that the conditions in the gut of the host act as a stimulus to the sporozoites within the oocyst to cause them to undergo excystation with little or no aid from the host.

The experiments in which a host that had just recovered from an infection

was reinfected by injection of oocysts is apparently not contradictory to the concept of mucosal immunity as postulated by Becker (35) in that the first infection did not involve enough of the intestinal mucosa to elicit a typical immune response. This behavior can be demonstrated in orally induced infections. Evidently, the second infection induced by injection of oocysts did involve the mucosa sufficiently to evoke a nearly typical immune response. However, the production of a few complete and moderate numbers of abnormal oocysts in the immune animals to which oocysts were administered orally or by injection is apparently atypical. Becker (1935) is of the opinion that the immune reaction is explainable on the basis of macrophage sensitization (which, as he stated, appears unlikely) or on the basis of the development of a mechanical reaction of the epithelial cells to prevent entrance of sporozoites. It may be that this reaction is not of the same unequivocal nature at borders of the mucosal cells other than the luminal. This could account for penetration by sporozoites of immune cells that would not normally be parasitized. Also, it is possible that the blocking reaction does not prevent penetration by infective endogenous stages. However, it seems necessary to postulate a cellular reaction which interferes with the development of at least some of the endogenous stages.

Another possibility exists which might account for the production of oocysts by the immune hosts. Roudabush (1937) found that the endogenous stages of *E. nieschulzi* are limited to the small intestine. Whether this limitation is due to regional differentiation of the mucosal cells or to regional environmental differences provided by the lumen of the small intestine, caecum, and colon is unknown. However, if an unfavorable lumenal environment normally limits the sporozoites and ensuing endogenous stages to the small intestine, then transport of sporozoites to mucosal cells of the caecum and colon would provide sites for development for the first-generation schizonts not normally available. Abnormal development and a high mortality rate might be expected in the ensuing infective endogenous stages because of exposure to an unfavorable environment. Thus, there would be a reduction in the numbers of gametocytes produced. Microgamete mortality might be extremely high and the large proportion of abnormal oocysts observed might represent unfertilized macrogametes. However, Roudabush (1937) feels that it is unlikely that fertilization is necessary for oocyst wall formation since the wall is nearly complete prior to the time that microgametes are available for fertilization. Hence, the abnormalities of the aberrant oocysts are probably not due to lack of fertilization. Further, it would seem that if the abnormal oocysts observed were due to localization in an unfavorable portion of the intestinal tract, then such oocysts should have been produced in all of the infections which were induced by injection. Such was not the case. The most likely explanation seems to be the development of a cellular reaction which interferes with the development of some of the endogenous stages.

The numbers of oocysts produced from an infection following injection are lower than those produced by an animal infected orally. This may be due to destruction of some of the injected sporozoites while in transit to the intestinal mucosa, to destruction of endogenous stages localized in an unfavorable portion of the gut, or to the localization of some of the sporozoites in epithelia other than the intestinal mucosa.

SUMMARY

Attempts to induce in vitro excystation of viable sporulated oocysts of *Eimeria nieschulzi* by exposing the oocysts to the action of digestive enzymes and digestive fluids, changes in oxidoreduction potentials, air pressure, and vacuum, and fresh blood were unsuccessful. The oocyst wall of *E. nieschulzi* is composed largely of polysaccharide and protein. When sporulated oocysts are injected into the blood stream, skeletal muscle, or the peritoneal cavity, they excyst and induce a typical subsequent infection in the host. The host may be reinfected in the same manner but, following recovery from the second infection, when these hosts were fed or injected with oocysts, they produced only a few complete oocysts and moderate numbers of degenerate or incomplete oocysts.

LITERATURE CITED

BECKER, E. R. 1935 The mechanism of immunity in murine coccidiosis. Am. J. Hyg. **12**: 389–404.

KRIJGSMAN, B. J. 1926 Wie werden im Intestinaltractus des Wirtstieres die Sporozoiten der Coccidien aus ihren Hüllen befreit? Arch. Prot. **56**: 116–127.

MONNÉ, L. AND HÖNIG, G. 1954 On the properties of the shells of the coccidian oocysts. Ark. Zool., Stockholm **7**: 251–256.

PATTILLO, W. H. AND BECKER, E. R. 1955 Cytochemistry of *Eimeria brunetti* and *E. acervulina* of the chicken. J. Morph. **96**: 61–96.

ROUDABUSH, R. L. 1937 The endogenous phases of the life cycles of *Eimeria nieschulzi, E. separata,* and *E. miyairii,* coccidian parasites of the rat. Iowa St. Coll. J. Sci. **11**: 135–163.

VAN DOORNINCK, W. M. AND BECKER, E. R. 1957 Transport of sporozoites of *Eimeria necatrix* in macrophages. J. Parasit. **43**: 40–44.

COCCIDIA FROM MINK IN BRITAIN

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There appears to be little information available regarding the species of coccidia occurring in farm mink (*Mustela vison*), and none at all from Britain. In North America, Kingscote (1934) and Levine (1948) have identified 4 species, namely, *Eimeria vison* Kingscote, 1934, *E. mustelae* Iwanoff-Gobzem, 1934, *Isospora bigemina* (Stiles, 1891) and *I. laidlawi* Hoare, 1927. Levine (1948) found all 4 species in a single farm mink from Illinois.

E. vison was originally described under the name of *"E. mustelae,"* the description being based on oocysts recovered from Canadian and United States mink which had died of coccidiosis. The name *"E. mustelae"* was later found to be preoccupied by *E. mustelae* Iwanoff-Gobzem, 1934, in view of which Kingscote (1935) renamed his species *E. vison*. Levine (1948), who also found this species in a mink, pointed to its morphological similarity to *E. sibirica* Yakimoff and Terwinsky, 1931, *E. irara* Carini and da Fonseca, 1938, and to *E. ictidea* Hoare, 1927, all of which occur in species of Mustelidae. He suggested that future experimental cross-infection studies might reduce them to synonymy. Kingscote (1934), on grounds of morphology and sporulation time, considered *E. ictidea* to be a distinct species.

E. mustelae was first described from the weasel (*Mustela nivalis*) in Kazakhstan (Iwanoff-Gobzem, 1934). In that paper the author's name was given as "Frau Tierärztin P. S. Iwanoff, Gobzem," but the generally accepted form appears to be "Iwanoff-Gobzem." Levine (1948) found *E. mustelae* in a mink and described it in much greater detail than was given by Iwanoff-Gobzem, but agreement between the 2 descriptions was not entirely satisfactory.

Isospora bigemina occurs in the dog, cat, fox, mink, and probably other closely related carnivores (Morgan and Hawkins, 1948). There are 2 distinct races of this species which are distinguishable by their size and tendency not to occur together. Specimens of the smaller race were recovered from a mink and described by Levine (1948).

The original description of *I. laidlawi* Hoare, 1927, was based on 35 to 40 oocysts recovered from the feces of ferrets in Britain. Levine (1948) found the same species in a mink in the United States. Although his description and measurements were based on only 5 oocysts, they were in sufficient agreement with those of Hoare to justify identification.

In the present study 200 mink from 42 farms in various parts of Britain were examined post mortem.

RESULTS

Coccidial oocysts were recovered from the intestinal contents of 8 mink, although death was not due to coccidiosis in any of these cases. In a survey of 896

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mink autopsies, which included most of the 200 animals in the present study, Head (1959) found coccidiosis to be the cause of death in only one case. *E. mustelae* and *I. bigemina* were not encountered in the present series, but *E. vison* was found in 3 cases and *I. laidlawi* in 5. Descriptions of the oocysts of the latter 2 species are given below, all measurements being expressed in microns.

Eimeria vison Kingscote, 1934

The 3 mink infected with this species originated on 3 different farms, and were 2, 5 and 6 months old respectively. Oocysts were confined to the posterior three-fifths of the intestine. They were usually ellipsoidal, sometimes ovoid, and occasionally sub-spherical. Levine (1948) described them as ellipsoidal, but Kingscote (1934) called them oval or egg-shaped. In the literature there is an unfortunate lack of consistency in the use of words to describe the shape of oocysts. In the present paper the following words are used in the senses defined by D'Arcy Thompson (1942), "ellipsoidal" meaning that the ends are equally rounded, and "ovoid" meaning "like a hen's egg . . . a little blunter at one end than the other."

No micropyle was visible. The oocyst wall was double, the outer layer being colorless and the inner layer a pale straw color. Kingscote (1934) described this color as yellow-brown, and Levine (1948) called it salmon pink, but thought that

FIG.1

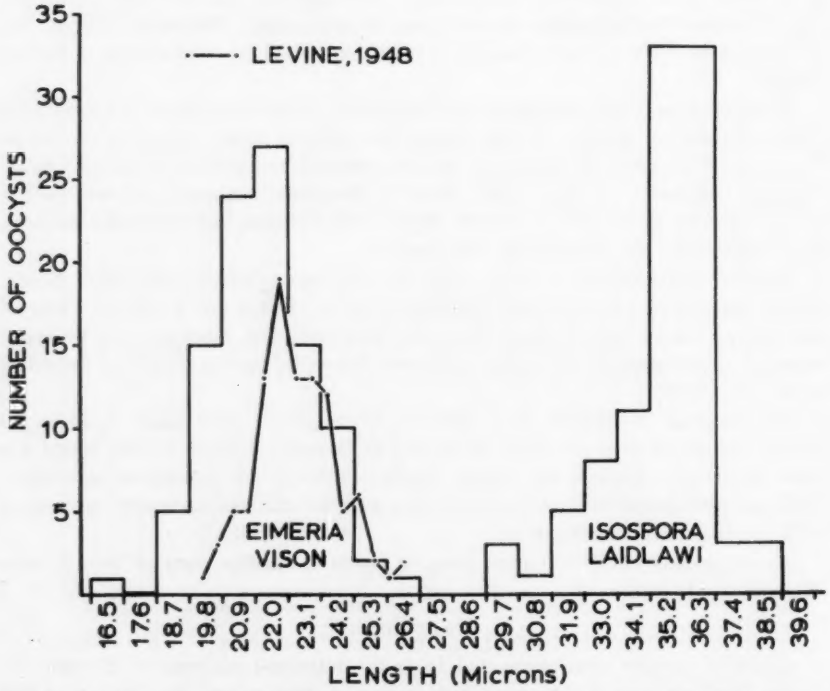


FIGURE 1. Frequency distribution of lengths of oocysts of *E. vison* and *I. laidlawi* from the mink compared with Levine's (1948) figures for *E. vison*.

this might be due to staining with haemolysed blood. One hundred sporulated oocysts were measured in the present study and were found to range in length from 16.5 to 26.4, mean 21.7, mode 22.0; and in width from 12.1 to 16.5, mean 14.6, mode 14.3. They had length: width ratios ranging from 1.2 to 1.9, mean 1.49, modes 1.35 and 1.55. This sample did not include any sub-spherical oocysts. The frequency distributions of the lengths and length: width ratios are graphically represented in figures 1 and 2, respectively, in which they are compared with Levine's (1948) figures for the same species. The length is given by Levine as 20 to 26, mean 22.8, and by Kingscote (1934) as 17 to 22.1, mean 20.3. It is noteworthy that while these ranges overlap by only about 2 microns, the range covered by the combined figures of these 2 authors agrees closely with our own.

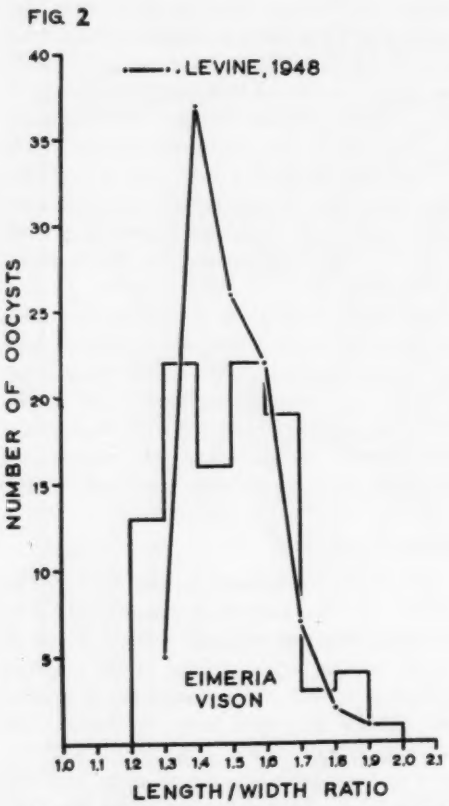
The sporulation time for *E. vison* was found to be about 5 days at room temperature. Levine (1948) found it to be 3 days and Kingscote (1934) 7 days. This inconsistency is presumably due to the differing (unrecorded) room temperatures. Each sporulated oocyst contained 4 sporocysts, each with 2 sporozoites. The various features of the sporulated oocysts agreed closely with the descriptions given by Kingscote (1934) and Levine (1948). In particular, the absence of a distinct oocyst residual body and the presence of a large granular sporocyst residual body were noted.

Isospora laidlawi Hoare, 1927

Of the 5 mink in which *I. laidlawi* was found, 3 originated on one farm. Their ages ranged from 6 months to several years. The numbers of oocysts present varied from few to very many, the latter cases being associated with macroscopic lesions in the intestine. As in the case of *E. vison,* oocysts were confined to the posterior three-fifths of the intestine. The oocysts were mostly ovoid, sometimes ellipsoidal, and occasionally subspherical. Hoare (1927) described them as "ovoid" and Levine (1948) as "ellipsoidal." No micropyle was visible. The oocyst wall was double, the outer layer being colorless. The inner layer was described by Hoare as "yellowish" and by Levine as "pale yellowish." In the present study the inner layer was found to be almost colorless, at most very pale yellow. It is suspected that this may be no more than an optical effect and that the inner layer is in fact colorless. This is not true of *E. vison* in which the color is quite distinct.

Measurement of 100 unsporulated oocysts gave lengths ranging from 29.7 to 38.5, mean 35.0, mode 35.7, and widths from 25.3 to 34.1, mean 29.3, mode 29.7. The length: width ratio varied from 1.04 to 1.52, mean 1.20, mode 1.19. The unsegmented zygote averaged 26.0 in diameter. Sporulation time at room temperature was 3 days. The frequency distributions of the lengths and length: width ratios are graphically represented in figures 1 and 3, respectively. Hoare (1927) found unsporulated oocysts to have lengths 32.0 to 36.8, mean 34.0 widths 27.2 to 30.4, mean 29.0, with a sporulation time of 4 days.

In the present study sporulation was commonly accompanied by a loss of rigidity of the oocyst wall, resulting in distortion of its shape, but 22 apparently undistorted specimens were 31.9 to 40.7 long, mean 35.9, 23.1 to 30.8 wide, mean 28.0, with length: width ratios 1.07 to 1.67, mean 1.29. The 5 sporulated oocysts described by Levine (1948) were 32 to 36 long, 26 to 27 wide with length: width ratios 1.2 to 1.3. The twin sporocysts in our oocysts were 18.7 to 27.5 long, mean 23.1 and 14.3 to 19.8 wide, mean 16.6. No oocyst residual body was observed, but



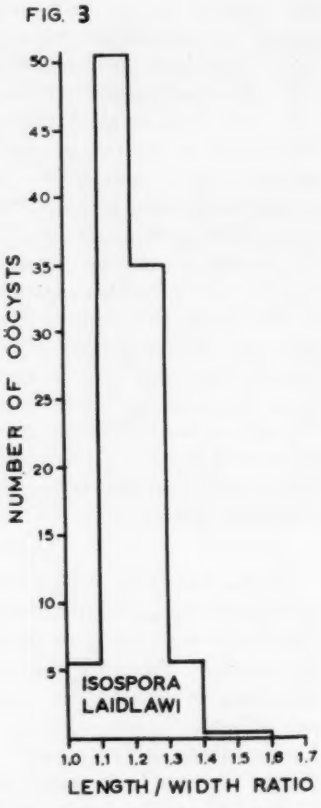


FIGURE 2. Frequency distribution of length: width ratios of oocysts of *E. vison* from the mink compared with Levine's (1948) figures.

FIGURE 3. Frequency distribution of length: width ratios of oocysts of *I. laidlawi* from the mink.

a large granular residual body was invariably present in each sporocyst in addition to 4 banana-shaped sporozoites. The residual body gradually disintegrated to form a number of refractile globules about 1 micron in diameter.

On one occasion an oocyst was observed which contained only 1 large sporocyst. The oocyst measured 35.2 by 27.7. Its shell was rigid and intact. The sporocyst measured 28.6 by 19.8 and contained 4 sporozoites measuring 19.8 by 5.5, and a residual body 16.5 in diameter.

SUMMARY

1. Two hundred mink originating on 42 farms in various parts of Britain were examined post mortem for coccidia.
2. *Eimeria vison* (Kingscote, 1934) was found in 3 mink from 3 farms, and *Isospora laidlawi* (Hoare, 1927) in 5 mink from 3 farms.
3. The oocysts are described in detail and the descriptions are compared with those of earlier authors.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to the Department of Pathology of this School, in particular to Mr. K. W. Head, B.Sc., M.R.C.V.S., for co-operation in providing the materials on which this study is based; also to Mr. R. S. Hood, F.I.B.P., F.I.S.T., for photographic processing of the graphs.

REFERENCES

CARINI, A. AND DA FONSECA, F. 1938 Sobre una nova *Eimeira* (sic) (*E. irara* n. sp.) parasita da *Tayra barbara*. Arch. Biol., S. Paulo **22:** 36.

HEAD, K. W. 1959 Vet. Rec. In press.

HOARE, C. A. 1927 On the coccidia of the ferret. Ann. Trop. Med. Parasit. **21:** 313–320.

IWANOFF-GOBZEM, P. S. 1934 Zum Vorkommen von Coccidien bei kleinen wilden Säugetieren. Deut. Tierärztl. Wschr. **42:** 149–151.

KINGSKOTE, A. A. 1934 *Eimeria mustelae* n. sp. from *Mustela vison*. J. Parasit. **20:** 252–253.

——— 1935 A note on the coccidia of the mink. J. Parasit. **21:** 126.

LEVINE, N. D. 1948 *Eimeria* and *Isospora* of the mink (*Mustela vison*). J. Parasit. **34:** 486–492.

MORGAN, B. B. AND HAWKINS, P. A. 1948 Veterinary Protozoology. Burgess Publishing Co., Minneapolis.

STILES, C. W. 1891 Note préliminaire sur quelques parasites. Bull. Soc. Zool. France **16:** 163–165.

THOMPSON, D'A. W. 1942 On Growth and Form. University Press, Cambridge.

YAKIMOFF, W. L. AND TERWINSKY, S. K. 1931 Die Coccidiose des Zobeltieres. Arch. Prot. **73:** 56–59.

RESEARCH NOTES

THE USE OF ANTICOAGULANTS AND CHLOROBUTANOL FOR THE RECOVERY OF ADULT SCHISTOSOMES FROM MICE.

In laboratory experiments on schistosomiasis it is often necessary to perfuse large numbers of infected animals. The techniques of Yolles et al (1947, J. Parasit. **33:** 419–426) and of Pan and Hunter (1951, J. Lab. and Clin. Med. **37:** 815–816) proved to be unduly time-consuming. Recently the author was introduced to the Perf-O-Suction Techniuqe as described briefly by Radke et al (1957, J. Parasit. **43** (supp.): 26–27). This proved to be superior to the other techniques for perfusing small animals to recover *Schistosoma mansoni*. However, as with the other procedures used, difficulty was experienced in removing adult specimens of *Schistosomatium douthitti*, especially from the smaller mesenteric blood vessels. When complete worm counts were required, it was necessary to examine these vessels under a dissecting microscope or a binocular eye-loupe and to tease the parasites out of them, a slow and tedious process.

Anticoagulants such as sodium or calcium citrate or heparin have been used in schistosome work (Stirewalt et al 1951, Am. J. Trop. Med. **31:** 57–82; Kagan, 1952, J. Inf. Dis. **91:** 147–158, and others). In addition to herapin or salts of citrate, collection of worms from the tissues was greatly facilitated by the use of an anaesthetic, namely chlorobutanol. Intraperitoneal injection of 7.5 mg of this substance in 0.5 ml distilled water resulted in considerable relaxation of the parasites thus allowing their easy removal from the vessels by the Perf-O-Suction technique. Furthermore, this dosage anaesthetized the mice, thus facilitating perfusion and eliminating the necessity of using ether.

The effectiveness and accuracy of this technique was ascertained by removing the viscera from the animal after perfusion and searching for remaining parasites in liver, intestine, and attached blood vessels. This was done by careful dissection and by squeezing the tissues between transparent plastic plates and examining them under the dissecting microscope. In a series of 46 perfusions only one parasite was encountered, an indication of the effectiveness of this technique.

This note is being published in the hope that this information will prove helpful to others who may be faced with a similar problem in their experimental work. The investigation was supported in part by a grant (E-1893) from the National Institutes of Allergy and Infectious Diseases, of the National Institutes of Health, U.S.P.H.S.—GEORGE W. HUNTER, III, *Department of Microbiology, College of Medicine, University of Florida, Gainesville, Florida.*

A NEW LOCALITY RECORD FOR *HETEROBILHARZIA AMERICANA* PRICE, 1929.

Price (1929, Proc. U. S. Nat. Museum, **75:** 1–39) described the male of the schistosome, *Heterobilharzia americana*, from the mesenteric veins of the bobcat, *Lynx rufus*. Price (1943, Proc. Helm. Soc. Wash. **10:** 85–87) later described the female and redescribed the male from specimens taken from a raccoon in Texas.

During the routine examination of a raccoon from Bladen County, North Carolina, on December 27, 1958, 15 males and 13 females of *H. americana* were discovered in the mesenteric vessels. A few of the best specimens were selected for study and comparison with the latest description by Price. Essentially our observations substantiate his descriptions, but the excellence of the material makes possible the addition of some details.

The average measurements in millimeters of 8 selected male specimens are as follows: length, 15.4; width, 1.00; oral sucker diameter, 0.45; acetabulum, 0.55; distance between acetabulum and gynecophoric canal, 0.61; number of testes, 60–84 (average 74). These figures approximate those given by Price (1929) for the worms taken from the bobcat, but the smaller measurements given for his raccoon specimens were probably caused by extreme contractions. This was the explanation of Price, and we are inclined to agree with him. Average measurements for 5 selected female specimens are: length, 14.3; width, 0.56; oral sucker, 0.10; acetabulum, 0.11; length of forebody to acetabulum, 0.46. These measurements are considerably greater than those given by Price (1943). This discrepancy, however, is explained on the basis of the contraction of specimens. Price stated that the intestinal ceca in several of the male specimens from the raccoon failed to unite posteriorly. Our specimens show much variation in this respect. Hardly any 2 worms are alike, and the ceca in all specimens unite at least once, and in some cases up to 4 separate unions are evident before the ceca terminate.

Although minor variations are evident, these schistosomes are obviously *Heterobilharzia americana* Price, 1929. This is the third report for this species and only the second report for the female worm. North Carolina is a new locality for this parasite. Present attempts are being made to determine the life cycle of this worm.—GROVER C. MILLER and REINARD HARKEMA, *Department of Zoology, North Carolina State College, Raleigh, North Carolina.*

EIMERIA AND *TYZZERIA* (PROTOZOA: EIMERIIDAE) FROM DEERMICE (*PEROMYSCUS* SPP.) IN ILLINOIS

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During the spring and summer of 1958, wild rodents were trapped in the vicinity of Sullivan, Illinois, as part of a leptospirosis survey under the direction of Drs. Deam H. Ferris and Lyle E. Hanson of the University of Illinois College of Veterinary Medicine and Dr. Rexford D. Lord, Jr. of the Illinois State Natural History Survey. When the rodents were brought to Urbana for examination, an opportunity to examine them for coccidia was afforded us. The present paper describes five species of coccidia including three new species found in 16 deermice (9 *Peromyscus maniculatus* and 7 *P. leucopus*).

MATERIALS AND METHODS

At the time of necropsy, the contents of the colon and cecum were placed in 2.5 percent potassium bichromate solution, mixed thoroughly, placed in a thin layer in a Petri dish, and kept at room temperature for a week or more in order to allow the coccidian oocysts to sporulate. The samples were then stored in a refrigerator. They were examined microscopically after sugar flotation, using a microscope with apochromatic objectives.

At the same time, segments of duodenum, jejunum, anterior ileum, posterior ileum and cecum were fixed in Zenker's fluid. They were later sectioned and stained with hematoxylin and eosin by Mrs. Marjorie B. Losch, and examined histologically for endogenous stages of the coccidia.

The hosts were identified by Dr. Ferris or by Dr. Donald F. Hoffmeister, Department of Zoology, University of Illinois.

RESULTS

Eimeria delicata n. sp.

Description. Oocysts (fig. 1) ellipsoidal. Oocyst wall very pale yellowish, smooth, composed of a single layer about 0.6 microns thick. Micropyle absent. Eleven sporulated oocysts measured 13 to 15 by 10 to 12 microns with a mean of 14.2 by 11.3 microns; length: width ratios ranged from 1.1 to 1.4 with a mean of 1.25. Oocyst polar granule present. Oocyst residuum absent. Sporocysts elongate ovoid with a rather pointed end bearing a tiny Stieda body, very thin-walled, 8 by 4 to 5 microns with a length:width ratio of about 1.9. Sporozoites oriented lengthwise in sporocysts. Some sporocyst residual granules ordinarily present, although they may sometimes be absent.

No endogenous stages were found in the intestine.

Host. Peromyscus maniculatus (deer mouse). *E. delicata* was found in 2 out of 9 animals examined. One of the mice was also infected with *Tyzzeria peromysci* (see below), while the second had no other coccidia.

Location. Intestinal contents.

Locality. The host animals were trapped April 20 and 21, 1958, respectively, on the same farm on the northwest edge of Sullivan, Illinois.

The following five species of *Eimeria* have been described from rodents of the genus *Peromyscus: E. peromysci* Levine, Ivens and Kruidenier, 1957, and *E. arizonensis* Levine, Ivens and Kruidenier, 1957, from *P. truei; E. eremici* Levine, Ivens and Kruidenier, 1957, from *P. eremicus,* all from Grand Canyon National

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Park, Arizona (Levine et al, 1957); *E. langebarteli* Ivens, Kruidenier and Levine, 1959, from *P. boylii* from Chihuahua, Mexico (Ivens et al, 1959); *E. carolinensis* von Zellen, 1959 (von Zellen, 1959), from *P. leucopus* from North Carolina.

E. delicata is much smaller than any of these except *E. carolinensis*. It differs further from *E. peromysci* and *E. eremici* in that its oocyst wall is composed of a single layer rather than two, in lacking an oocyst residuum, and in the shape and size of its sporocysts. It differs further from *E. arizonensis* in that its oocyst wall is not lined by a membrane, in lacking an oocyst residuum, and in the shape and size of its sporocysts. It differs further from *E. langebarteli* in oocyst and sporocyst shape, in the shape and position of the sporozoites and in the nature of the sporocyst residual granules; the sporozoites of *E. langebarteli* are elongate and curled inside the sporocysts, with many small granules so closely adherent to them that it was not possible to be sure whether they were inside or outside the sporozoite membrane. It differs from *E. carolinensis* in having a thin, pale yellowish single-layered wall rather than a thick one with an outer, colorless layer and an inner dark brown one.

Eimeria roudabushi n. sp.

Description. Oocysts (fig. 2) ellipsoidal. Oocyst wall smooth, almost colorless, composed of a single layer about 1.3 microns thick at the sides, grading to about 0.9 microns thick at the ends. Micropyle absent. Twenty sporulated oocysts measured 20 to 26 by 17 to 20 microns with a mean of 22.2 by 18.6 microns; their length:width ratios ranged from 1.1 to 1.3, with a mean of 1.19. Oocyst polar granule present. Oocyst residuum atypical, composed of a small amount of cobwebby-appearing material at both ends of the oocyst. Sporocysts ovoid, thin-walled, with small Stieda body. Sporocysts 12 to 13 by 8 microns with a length:width ratio of 1.6 to 1.7. Sporocyst residuum large, compact. Sporozoites colorless, lying lengthwise, head to tail, in sporocysts, but so long that they are folded back upon themselves.

No endogenous stages were found in the intestine.

Host. Peromyscus leucopus (deer mouse). This species was found in 1 out of 7 animals examined.

Location. Intestinal contents.

Locality. The host animal was trapped July 7, 1958, on a farm northwest of Sullivan, Illinois.

Six species of *Eimeria* have been described from rodents of the genus *Peromyscus* (see under *E. delicata* above). *E. roudabushi* differs from them all in the fact that the oocyst wall is thinner on the ends that at the sides. Its oocyst residuum is markedly different in appearance from those of the other three species which have this structure. It differs further from *E. peromysci* in the shape and size of its sporocysts and in having a single-layered, smooth oocyst wall. It differs further from *E. arizonensis* in the shape of its sporocysts and in not having a membrane lining the oocyst wall. It differs further from *E. eremici* in the shape of its sporocysts and in having a single-layered oocyst wall. It differs further from *E. langebarteli* in having an oocyst residuum, in the shape and size of its sporocysts, and in the nature of its sporocyst residuum. It differs further from *E. delicata* in size, in having an oocyst residuum, and in the shape and size of its sporocysts. It differs further from *E. carolinensis* in having a single-layered rather than a two-layered oocyst wall, in oocyst size, and in the appearance of its sporozoites.

This species is named in honor of Dr. Robert L. Roudabush, Rochester, N. Y.

Eimeria arizonensis Levine, Ivens and Kruidenier, 1957

This species was first described from *Peromyscus truei* in Grand Canyon National Park, Arizona. In the present survey, the same species or closely similar ones were found in *P. manic-*

ulatus and *P. leucopus*. Since there were minor differences between the forms from these three hosts, those from the Illinois deermice are described separately below.

Description. Form from *P. maniculatus*. Oocysts (fig. 3) ellipsoidal. Oocyst wall light yellowish, slightly to moderately pitted, occasionally smooth, composed of a single layer (confirmed by crushing the oocyst) 1.3 to 1.7 microns thick. Micropyle absent. Forty sporulated oocysts measured 22 to 29 by 18 to 23 microns with a mean of 26.1 by 20.8 microns; their length: width ratios ranged from 1.1 to 1.3, with a mean of 1.24. Oocyst polar granule present. Oocyst residuum composed of a cluster of large, homogeneous granules or globules 2 to 5 microns in diameter. Sporocysts lemon-shaped, with prominent Stieda body. Sporocyst wall 0.4 microns thick. Twenty-three sporocysts measured 11 to 13 by 7 to 9 microns with a mean of 12.2 by 8.0 microns; their length:width ratios ranged from 1.3 to 1.7, with a mean of 1.56. Sporozoites homogeneous, colorless, lying lengthwise in sporocysts. Sporocyst residuum composed of coarse granules filling the space between the sporozoites.

No endogeneous stages of this form were found in the duodenum, jejunum or cecum. A few microgametocytes, macrogametes and young oocysts were seen in the epithelial cells of the villi of the anterior ileum. The macrogametes had a single layer of eosinophilic plastic granules. Their relation to the host cell nucleus was uncertain. They measured approximately 19 by 14 microns when mature, but were shrunken. The young oocysts measured approximately 22 by 16 microns. A few macrogametes were also seen in the posterior ileum.

Form from *P. leucopus*. Oocysts (fig. 4) ellipsoidal to broadly ellipsoidal. Oocyst wall pale yellowish to yellowish, occasionally almost smooth, but usually more or less pitted, composed of a single layer (confirmed by crushing the oocyst) 1.0 to 1.2 microns thick. Micropyle absent. Sixty-five sporulated oocysts from two host individuals measured 20 to 25 by 17 to 21 microns, with a mean of 22.4 by 19.4 microns; their length:width ratios ranged from 1.1 to 1.3, with a mean of 1.16. One or 2 oocyst polar granules present. Oocyst residuum present, usually a single, large, waxy-appearing globule about 4 microns in diameter. Sporocysts lemon-shaped to rather ovoid, with prominent Stieda body. Sporocyst wall rather thick. Twenty-two sporocysts measured 12 to 14 by 7 to 8 microns, with a mean of 12.6 by 7.2 microns; their length:width ratios ranged from 1.6 to 1.9 with a mean of 1.75. Sporocyst residuum composed of more or less scattered coarse granules. Sporozoites colorless, lying lengthwise, head to tail, in sporocysts.

A moderate number of macrogametes, microgametocytes and oocysts were found in the epithelial cells of the tips and sides of the villi, and very rarely in the crypts of the jejunum. Fewer parasites were found in the anterior ileum, still fewer in the posterior ileum, and only a single macrogamete in the duodenum. No endogenous stages were seen in the cecum. All stages were present both above and below the host cell nuclei; the older forms tended to lie below them. The macrogametes ranged in size up to about 14 by 10 microns, and had a single layer of eosinophilic plastic granules. The microgametocytes ranged in size up to about 15 by 8 microns. The oocysts ranged in size up to about 19 by 14 microns.

A very few schizonts were seen in the jejunum, and a single one was found in a detached piece of villus in the posterior ileum. They lay beneath the host cell nuclei in the epithelial cells at the sides of the villi, but too few were seen to be sure that this is their only location. They measured 9 to 12 by 7 to 9 microns. Most of them seemed to contain about 8, or perhaps a few more, sausage-shaped merozoites about 1 micron in diameter, but one schizont contained perhaps 16 to 24; these may possibly have belonged to 2 different generations. There was no schizont residuum.

Locality. The form from *P. maniculatus* was found in 1 of 9 deermice. The host animal was trapped April 20, 1958, on the same farm on the northwest edge of Sullivan, Illinois, where *E. delicata* had been found. The host animal was also infected with *Tyzzeria peromysci* (see below).

The form from *P. leucopus* was found in 2 of 7 deermice. The host animals were trapped June 30, 1958, in a pasture 6 miles north of Sullivan, Illinois. One of the host animals was also infected with *Tyzzeria.*

The form from *P. maniculatus* differs from *E. arizonensis* from *P. truei* only in that its oocyst wall is thicker and is ordinarily more or less pitted, in that it lacks a membrane lining the oocyst wall, and in that its oocyst residuum is composed of a cluster of homogeneous globules rather than a single large one. The form from *P. leucopus* differs from *E. arizonensis* from *P. truei* only in that its oocyst wall is more or less pitted and in that it lacks a membrane lining the oocyst wall. The oocysts of the form from *P. leucopus* differ from those of the form from *P. maniculatus* described above only in having a thinner oocyst wall and in that the oocyst

residuum is composed of a single, large, homogeneous globule rather than a cluster of them. Those endogenous stages which were seen did not appear to differ morphologically. However, none was seen in the jejunum of *P. maniculatus*, while they were most common in this location in *P. leucopus*. Since so few were found in the former, this should not be considered a valid difference. The above differences do not appear sufficient to justify the establishment of new species, altho it is not impossible that future research may reveal differences in the endogenous stages, location in the host or host-parasite relations which would do so.

Tyzzeria peromysci n. sp.

This species was found in both *Peromyscus maniculatus* and *P. leucopus*. Since there were minor differences between the forms from the two hosts, they are described separately below.

Description. Form from *P. maniculatus* (type host): Oocysts (fig. 5) ellipsoidal. Oocyst wall colorless to very pale yellowish, smooth, composed of a single layer about 0.6 microns thick. Micropyle absent. Forty sporulated oocysts from two host animals measured 11 to 14 by 9 to 11 microns, with a mean of 12.6 by 10.1 microns; their length:width ratios ranged from 1.1 to 1.4, with a mean of 1.24. One or 2 polar granules present. Sporozoites banana-shaped, crescent-shaped or lanceolate, about 3.5 microns wide and 9 to 11 microns long. Sporozoites clustered together in a ball without orientation in any particular direction, sometimes with 1 or 2 sporozoites separated from the others. Sporozoites often with fine granules in cytoplasm except in central region where the nucleus is located; sporozoites sometimes without granular cytoplasm. No oocyst residuum was seen, but it is possible that a small one may have been concealed inside the ball of sporozoites.

No endogenous stages were found in the intestines of the two host animals examined.

Form from *P. leucopus*. Oocysts ellipsoidal. Oocyst wall very pale yellowish, smooth, composed of a single layer about 0.6 microns thick. Micropyle absent. Twenty sporulated oocysts measured 14 to 17 by 11 to 12 microns with a mean of 15.2 by 11.2 microns; their length: width ratios ranged from 1.3 to 1.5 with a mean of 1.36. One or 2 polar granules present. Sporozoites banana-shaped, crescentic or lanceolate, clustered together in a ball without orientation in any particular direction, sometimes with 1 or 2 sporozoites separated from the others. A small, compact oocyst residuum 3 to 4 microns in diameter was seen in some oocysts in which the sporozoites were sufficiently well separated, and one may have been present but hidden by the sporozoites clustered around it in most or all of the other oocysts.

No endogenous stages were found in the intestine.

Location. Intestinal contents.

Locality. The form from *P. maniculatus* was found in 2 out of 9 deermice trapped April 20 and 21, 1958, on a farm on the northwest edge of Sullivan, Illinois. One animal was also infected with *Eimeria delicata* and the other with *E. arizonensis*. The form from *P. leucopus* was found in 1 out of 7 deermice. The host animal was trapped June 30, 1958, in a pasture 6 miles north of Sullivan. It was also infected with *E. arizonensis*.

The form from *P. maniculatus* differs from that from *P. leucopus* only in that it is slightly smaller and broader and in that no oocyst residuum was seen in it. However, as mentioned above, it is not impossible that an oocyst residuum may have been present in the center of the ball of sporozoites. These differences do not appear sufficient to justify the establishment of separate species, although it is not impossible that future research may reveal differences in the endogenous stages, location in the host or host-parasite relations which would do so.

It is worth noting that no endogenous stages were found in the intestinal tracts of the 3 host animals. It is uncertain whether this means that too few parasites were present to be found, that they occur in some other location, or perhaps they are actually parasites of some invertebrate which the deermice ate.

The genus *Tyzzeria* is characterized by oocysts which contain 8 naked sporozo-

ites, but no sporocyst. Four species have been named, 3 in birds and 1 in a snake. *T. perniciosa* Allen, 1936 (Allen, 1936), was described from the domestic duck, *Anas platyrhynchos,* on Long Island, New York. *T. alleni* Chakravarty and Basu, 1947 (Chakravarty and Basu, 1947), was described from the cotton teal, *Cheniscus* (syn., *Nettapus*) *coromandelianus,* in India. *T. anseris* Nieschulz, 1947 (Nieschulz, 1947), was described from the domestic goose, *Anser anser,* in the Netherlands. This species was later found in four subspecies of the Canada goose, *Branta canadensis,* and in the Atlantic brant, *B. bernicla,* white-fronted goose, *Anser albifrons,* blue and snow geese, *A. caerulescens ssp.,* Ross's goose, *A. rossi,* and whistling swan, *Olor columbianus* (see Hanson, Levine and Ivens, 1957, for references). *T. natrix* (Matubayasi, 1936) Matubayasi, 1937 (Matubayasi, 1937), was described from the water snake, *Natrix tigrina,* in Japan.

There are few oocyst characters which can be used to differentiate species of the genus *Tyzzeria.* However, *T. peromysci* differs from the three avian species and *T. natrix* in lacking a prominent oocyst residuum. In addition, the sporozoites of the avian species lie loosely around the residuum. *T. peromysci* differs from *T. natrix* in having ellipsoidal rather than spherical oocysts. Even without these morphological differences, however, the fact that the host of this *Tyzzeria* is a mammal would justify establishing a new species for it.

SUMMARY

In a survey of the coccidia of deermice in central Illinois, *Eimeria delicata* n. sp. was found in 2 of 9 *Peromyscus maniculatus, E. roudabushi* n. sp. in 1 of 7 *P. leucopus, E. arizonensis* Levine, Ivens and Kruidenier, 1957, in 1 *P. maniculatus* and 2 *P. leucopus* (both new host records), and *Tyzzeria peromysci* n. sp. was found in 2 *P. maniculatus* and 1 *P. leucopus.* This is the first report of *Tyzzeria* from mammals.

LITERATURE CITED

ALLEN, E. A. 1936 *Tyzzeria perniciosa* gen. et sp. nov., a coccidium from the small intestine of the Pekin duck, *Anas domesticus* L. Arch. Prot. **87:** 262–267.

CHAKRAVARTY, M. AND BASU, S. P. 1947 On a new coccidium, *Tyzzeria alleni* n. sp., from the intestine of the bird cotton-teal. Sci. and Culture (Calcutta) **12:** 106.

HANSON, H. C., LEVINE, N. D. AND IVENS, V. 1957 Coccidia (Protozoa: Eimeriidae) of North American wild geese and swans. Can. J. Zool. **35:** 715–733.

IVENS, V., KRUIDENIER, F. J. AND LEVINE, N. D. 1959 Further studies of *Eimeria* (Protozoa: Eimeriidae) from Mexican rodents. Trans. Ill. State Acad. Sci. **51:** 53–57.

LEVINE, N. D., IVENS, V. AND KRUIDENIER, F. J. 1957 New species of *Eimeria* from Arizona rodents. J. Prot. **4:** 80–88.

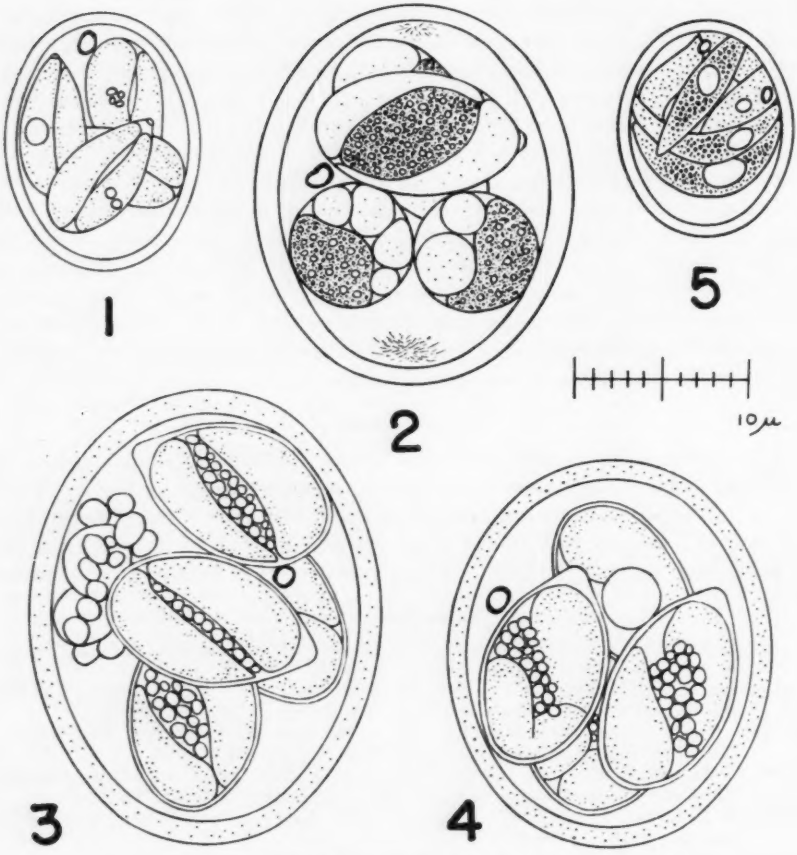
MATUBAYASI, H. 1936 On a new coccidia *Koidzumiella natrix* n. g., n. sp., parasitic in Japanese snakes; with some remarks on two ophidian coccidia of the genus Caryospora and Isospora. Keio Igaku, Tokyo **16:** 1719–1729.

———1937 Studies on parasitic protozoa in Japan. II. Coccidia parasitic in snakes; with special remarks on *Tyzzeria* (*Koidzumiella*) *natrix,* parasitic in *Natrix tigrina.* Annot. Zool. Jap. **16:** 255–275.

NIESCHULZ, O. 1947 Eine neue Kokzidienart bei der Hausgans. Zbl. Bakt. 1. Abt. Orig. **152:** 74.

VON ZELLEN, B. W. 1959 *Eimeria carolinensis* n. sp., a coccidium from the white-footed mouse, *Peromyscus leucopus* (Rafinesque). J. Prot. **6:** 104–105.

PLATE I



EXPLANATION OF PLATE 1

FIGURE 1. Oocyst of *Eimeria delicata* n. sp. from *Peromyscus maniculatus*, ×2600.
FIGURE 2. Oocyst of *Eimeria roudabushi* n. sp. from *Peromyscus leucopus*, ×2600.
FIGURE 3. Oocyst of *Eimeria arizonensis* from *P. maniculatus*, ×2600.
FIGURE 4. Oocyst of *Eimeria arizonensis* from *P. leucopus*, ×2600.
FIGURE 5. Oocyst of *Tyzzeria peromysci* n. sp. from *P. maniculatus*, ×2600.

TWO NEW FLEAS (HYSTRICHOPSYLLIDAE) FROM THE KANGAROO RAT AND POCKET MOUSE

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While examining a series of fleas collected in the Big Bend National Park in 1955 by Dr. Sherman Minton, Department of Microbiology, Indiana University School of Medicine, a male *Meringis* from a kangaroo rat was found which appeared to be distinct from related species. Mr. R. D. Porter, assigned to the Park to conduct ecological studies on rodents in pursuit of his Doctor of Philosophy degree at Texas Agricultural and Mechanical College, College Station, Texas, kindly examined a number of kangaroo rats and other rodents, and provided us with a long series of the *Meringis* in which we were currently interested, and a second species also believed to be undescribed.

Acknowledgment is also made of the taxonomic assistance given by Drs. Phyllis Johnson, U. S. National Museum, Washington, D. C., and G. F. Augustson, Manager, Madera County Abatement District, Madera, California.

Meringis vitabilis n.sp. (Plate I)

MALE. Head: Cephalic margin of head evenly rounded, without frontoclypeal tubercle. Preantennal region with 3 relatively large dermal pores along cephalic margin and 3 smaller ones along ventral margin; 2 rows of 4 setae each, the caudal or ocular row the larger, 2 or 3 microsetae intercalated between setae in each row; ventrad of reduced, lightly pigmented eye an irregular row of about 4 microsetae. Scape of antenna with a small basal seta and 4 on posterior half, none of which reach caudal margin. Genal process bluntly acuminate distally, outer genal spine superimposed on basal three-fourths of the narrower but longer inner genal spine. Three short, stout setae in front of the base of the 4-segmented maxillary palpi; maxillary lobe acuminate distally, extending from one-half to full length of the 3d labial palpal segments; labial and maxillary palpi subequal, reaching about three-fourths the length of the fore coxae; maxillary laciniae wider than the epipharyngeal stylet, almost one-half the diameter and about the same length as the labial palpi. Eleven or 12 small setae along ventral margin of the postantennal region, the distal 2 the largest; the 3 rows of postantennal setae arranged 4-4-5, with the ventral bristle the longest in each row; in the 3d row 4 small setae interpolated among the larger ones.

Thorax: Pronotal comb of 7 spines per side, preceeded by a row of 6 small setae alternating with 6 large bristles. Mesonotum with numerous small setae on cephalic margin followed by a row of about 7 setae, a row of 6 or 7 larger ones, a row of 5 still larger setae with small ones intercalated between them, and a caudal row of 2 or 3 pseudosetae. Mesepisternum with a small median seta and 2 longer caudal ones; mesepimeron with 3 cephalic setae in an irregular row followed by a row of 2 larger ones. Metanotum with a single dorsomesial seta followed by a row of 5 medium sized setae and a row of 5 or 6 longer setae alternating with small ones. Lateral metanotal area with a row of 2 large and 1 small setae; metepisternum with a large and a very small seta dorsomesially and a row of 2 or 3 microsetae running parallel with dorsal and ventral margins; 3 rows of setae on metepimeron which are variable in number, 3(4)-4(3)-1(2).

Legs: Profemora with about 13 lateral and submarginal setae, meso- and metafemora with no lateral setae and 7 or 8 submarginal ones; fore tibia with 10 subequal spines on or near margin, a verticle row of 5 small median setae and 2 long lateral setae, the most ventrad approximately the length of the tibial segment. Metacoxa with 5 to 7 spiniforms in an oblique row to horizontal plane of the body. Last tarsal segment of each leg with 4 pairs of lateral plantar bristles plus a median basal pair; pretarsal claws long, about two-thirds the length of the distal tarsal segment.

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Abdomen: Sternum of 2d abdominal segment with 1 seta; sterna 3 to 7 with a row of 3 or 4 setae, sometime preceded by a single median seta. Tergum 1 with 2 setae followed by a row of 5 or 6 moderate-sized setae and a row of a similar number of large setae with 5 fine ones intercalated among them; terga 2 to 7 with 2 rows of 6 to 8 setae, the caudal row composed of large ones. Terga without apical spinelets. The usual 3 antepygidial bristles, the middle 1 twice the length of the others.

Modified Abdominal Segments: Eighth tergum reduced, with 5 to 7 setae in an irregular row, occasionally a seta cephalad to row. Eighth sternum with dorsocaudal margin convex, caudal margin broadly concave, bluntly rounded at juncture with ventral margin; 8th sternum with an irregular row of 4 (3) large setae along ventral margin, plus 1 or 2 setae dorsad to this row. Ninth sternum with distal arm over twice the length of proximal arm; cephalic margin of distal arm with median convexity producing shallow basal and distal sinuses; apex of distal arm not acutely produced but somewhat rounded, with a small subapical spiniform and a seta about one-half as thick and twice as long as the spiniform; caudal margin with 2 larger spiniforms toward apex, the lower one the thickest and most heavily pigmented; usually a small seta between the spiniforms and an additional 5 to 7 following them; proximal arm at apex as the head of a raptorial bird, anterior margin fairly straight, posterior margin convex distally and remainder evenly concave.

Dorsal margin of immovable process of clasper divided into 2 definite lobes; the larger posterior lobe with 3 thin setae, the anterior lobe with a large and a smaller bristle at apex; caudal margin with a shallow subapical sinus followed by a broad convexity except for a slight indentation at the level of the single acetabular bristle. Movable finger extending well distad of immovable process, rounded at apex, and slightly over twice as long as its greatest width; a single small setae on dorsocephalic margin plus about 10 microsetae along and near remainder of the cephalic margin, several more of these microsetae scattered over the remainder of the movable process; about 13–18 thin marginal and submarginal setae on caudal margin which is evenly convex except for a slight sinus at dorsocaudal margin. Manubrium long and narrow, about 8 times as long as its greatest basal width, sides subparallel, tapering gradually from base to dorsad-directed tip.

Aedeagal apodeme shorter than aedeagus proper, widest toward distal end, produced at apex in a short, chitinized, upward projecting process. Lateral lobes of aedeagus long and thin, little broader at apex than base; crochets extending beyond median dorsal lobes, broadest at base, caudal half of dorsal margin tapering rapidly to subacuminate; sclerotized inner tube flared at apex. Penis rods short and uncoiled.

FEMALE. Caudal margin of 7th sternum divided into 2 subequal lobes by a distinct, although shallow sinus; 7th sternum with a row of 5 to 7 large setae, preceded by a less regular row of 4 to 7 smaller ones. Anal stylet approximately 3 times as long as basal width, with a long apical and 2 (3) minute subapical setae; 8th sternum tapering to bluntly acuminate distal end which bears 2 or 3 thin setae. Eighth tergum with a short dorsocephalic row of about 4 setae and an irregular row of some 15 longer ones extending entire width of plate, plus about 5 still larger setae on lower half and 15 or 16 subequal setae on caudal margin; caudal margin with prominent, acuminate dorsal and ventral lobes. Body of spermatheca with concave dorsal margin and convex ventral margin, arm mangled, longer than body.

Holotype Male: Ex *Dipodomys merriami,* Brewster Co., Tex., Feb. 24, 1958, coll. R. D. Porter. Deposited in U. S. National Museum.

Allotype Female: Ex *D. merriami,* Brewster Co., Tex., Feb. 19, 1958, coll. R. D. Porter. Deposited in U. S. National Museum.

Paratypes: 1 male ex kangaroo rat, Brewster Co., Tex., March 1955, coll. Sherman Minton; 1 male and 4 females ex *D. merriami,* Brewster Co., Tex., Feb. 19, 1958, coll. R. D. Porter; 2 males and 12 females ex *D. merriami,* Brewster Co., Tex., Feb. 24, 1958, coll. R. D. Porter, 2 males and 2 females ex *D. merriami,* Brewster Co., Tex., Feb. 26, 1958, coll. R. D. Porter; 1 female ex *D. merriami,* Brewster Co., Tex., Jan. 28, 1958, coll. R. D. Porter.

This flea belongs to the group of *Meringis* characterized by the absence of a proximal ventral lobe on the 9th sternum in the male and the subcylindrical sperm atheca in the female which includes *M. dipodomys* Kohls, *M. deserti* Augustson, *M. arachis* (Jordan), *M. rectus* Morlan, *M. bilsingi* Eads and Menzies, *M. nidi* Williams and Hoff and *M. altipecten* Traub and Hoff. This species can be separated from the closely related *dipodomys* and *deserti* by distinctive male genitalia. In *vitabilis* the movable finger of the clasper is broader in proportion to its height than in these 2 species and less broadly rounded apically; the lateral lobes of the aedeagus

in *vitabilis* are thin and approximately the same width throughout while they are much broadened basally in *dipodomys* and *deserti.* The crochets of *vitabilis* are also distinctively shaped.

Meringis agilis n. sp. (Plate II)

MALE. Head: Preantennal region of head with 4 setae in 1st row and 3 larger ones in 2d row; microsetae intercalated among both rows of setae with an irregular caudal row of 4 or 5 microsetae. Postantennal region with setae arranged 3-5-5 plus a row of 7 to 12 small setae along the ventral margin. Labial palpi extending about three-fourths of the length of the fore coxae.

Thorax: Pronotal comb of 7 spines per side, preceded by a row of 7 large bristles alternating with an equal number of smaller ones. Mesonotum well provided with scattered setae on anterior portion, followed by a row of 7 setae, a row of 6 larger ones with small setae intercalated between them and a pseudoseta toward the ventrocaudal margin; mesepisternum with 3 to 5 subequal setae but usually with a small median seta and an irregular caudal row of 3 setae; mesepimeron with a cephalic row of 4 subequal setae and a caudal row of 2 larger ones. Metanotum with a single seta followed by a row of 5 or 6 medium sized setae and a row of 6 or 7 larger setae alternating with small ones.

Modified Abdominal Segments: Eighth sternum with caudal margin sinuous, broadly concave; juncture of caudal and ventral margins bluntly rounded; ventral margin concave toward apical third; 8th sternum with an irregular row of 5 (4) setae along ventral margin and 1 or 2 setae dorsad of this row. Ninth sternum with distal arm twice the length of the proximal arm; cephalic margin of distal arm with median convexity producing basal and distal sinuses; apex of distal arm acutely produced, with a small apical spiniform and a longer seta; setation of caudal margin of 9th sternum distal arm variable, usually with 2 spiniforms toward apex, the lower being the heaviest and darkest, and several setae following them.

Dorsal margin of immovable process of clasper divided into 2 lobes by a shallow sinus; caudal margin concave, becoming convex before single acetabular bristle. Movable finger of clasper two and one-half times as high as greatest width, extending well beyond the immovable process. Finger with 15 to 18 thin bristles along caudal margin, 12 to 14 small setae on cephalic margin and remainder of the movable process. Manubrium long and narrow, about 6 times as long as its greatest basal width.

Crochets as figured, usually extending about as far as lateral and median dorsal lobes of aedeagus. Lateral lobes wide at base, tapering to subacute apex. Sclerotized inner tube slightly flared at apex. Penis rods short and uncoiled.

FEMALE. Caudal margin of 7th sternum divided into 2 lobes by a shallow sinus; lateral setation of seventh sternum variable, posterior row usually of 5 or 6 large setae (4 to 7) and anterior row usually of 3 or 4 smaller ones (1 to 5). Anal stylet less than twice as long as basal width, with 2 minute subapical setae in addition to the long apical seta. Eighth tergum with 4 or 5 irregularly placed setae caudad of antepygidial bristles, followed by a row of 12–15 setae extending entire width of plate and a row of some 12 subequal setae on caudal margin. Eighth sternum with 3 to 5 short apical setae. Dorsal anal lobe with 7 lateral setae and a dorsal marginal row of setae. Ventral anal lobe with 2 or 3 mesial setae and a thick ventromarginal row of around 12 heavy setae. Spermatheca body with dorsal margin concave and ventral margin fairly straight; arm about one-third the thickness of body and somewhat longer.

Holotype Male: Ex *Perognathus merriami,* Brewster Co., Tex., Feb. 20, 1958, coll. R. D. Porter.

Allotype Female: Ex *Perognathus nelsoni,* Brewster Co., Tex., Feb. 12, 1959, coll. R. D. Porter.

Paratypes: 1 male and 2 females, ex *Perognathus nelsoni,* Brewster Co., Tex., Feb. 25, 1958, coll. R. D. Porter; 13 males and 5 females ex *Perognathus nelsoni,* Brewster Co., Tex., Feb. 12, 1959, coll. R. D. Porter; 1 male and 1 female, ex *Perognathus penicillatus,* Brewster Co., Tex., Feb. 20, 1959, coll. R. D. Porter; 1 male ex *Perognathus merriami,* Brewster Co., Tex., Feb. 9, 1959, coll. R. D. Porter.

Due to the large series of *agilis* which have been taken from pocket mice, it is assumed that *Perognathus* is the normal host. Most of the *Meringis* commonly parasitize kangaroo rats, but this flea has not been collected from them even though they are prevalent in the type locality. As with *vitabilis,* this flea belongs to the group of *Meringis* which possesses a subcylindrical spermatheca in the female and lacks a proximal ventral lobe on the 9th strnum in the male. With *agilis* the more

acutely produced apex of the movable finger of the male clasper and the broad lateral lobes of the aedeagus which taper toward their apexes readily separate the species from *vitabilis*. In *dipodomys* the sinus of the dorsal margin of the immovable process of the male clasper is deep, forming a much larger posterior lobe than is the case with *agilis*. The movable finger of the male clasper is much less broadly rounded at the apex of *agilis* than in *dipodomys* and *deserti*.

SUMMARY

The following two new species of fleas have been described from rodents captured in the Big Bend National Park (Texas): (1) *Meringis vitabilis* taken from *Dipodomys merriami*, kangaroo rat; and (2) *M. agilis*, taken from *Perognathus merriami*, *P. nelsoni*, and *P. penicillatus*, pocket mice.

Explanation of Plates

Plate I.

A, *Meringis vitabilis*, male head; B, *M. vitabilis*, male modified abdominal segments; C, *M. vitabilis*, male process and movable finger of clasper; D, *M. vitabilis*, apex of male aedeagus; E, *M. vitabilis*, female seventh sternum.

Plate II.

A, *Meringis agilis*, male modified abdominal segments; B, *M. agilis*, apex of male aedeagus; C, *M. dipodomys*, male modified abdominal segments; D, *M. agilis*, female seventh sternum; E, *M. deserti*, male modified abdominal segments.

PLATE I

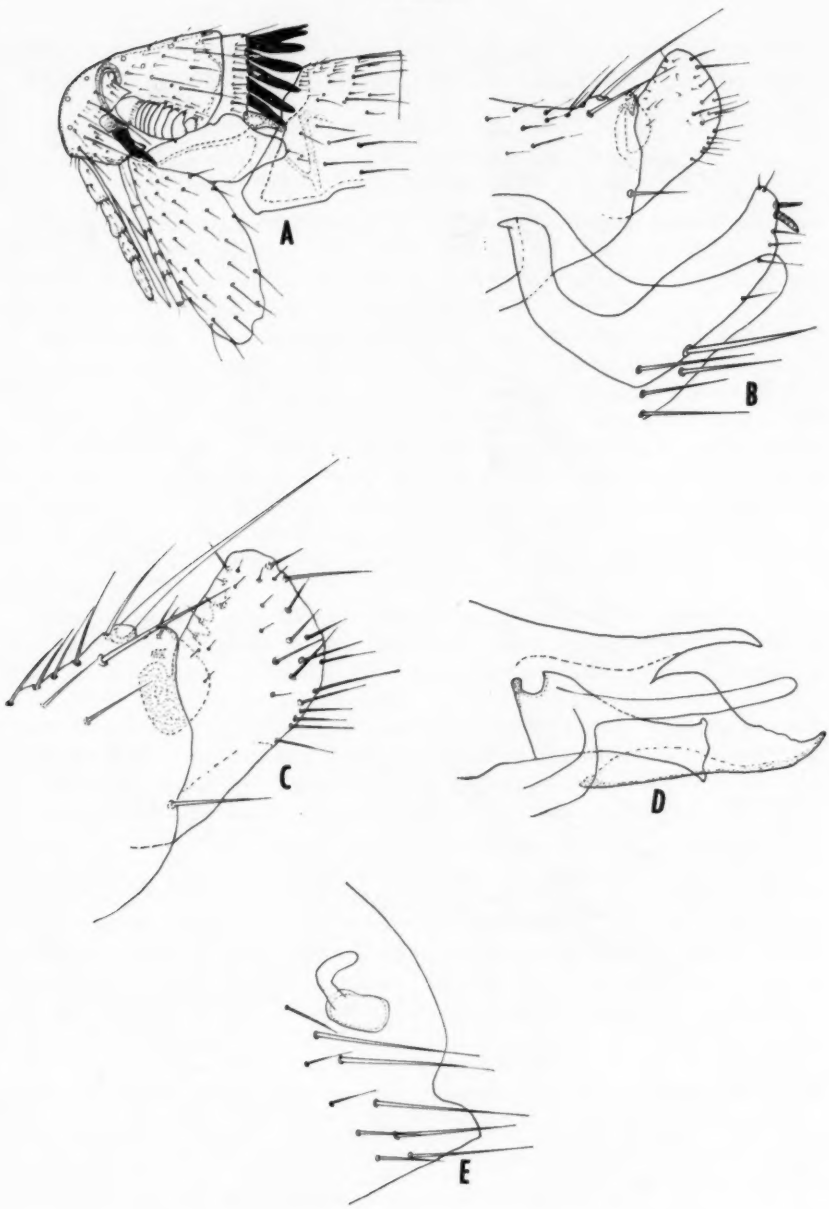
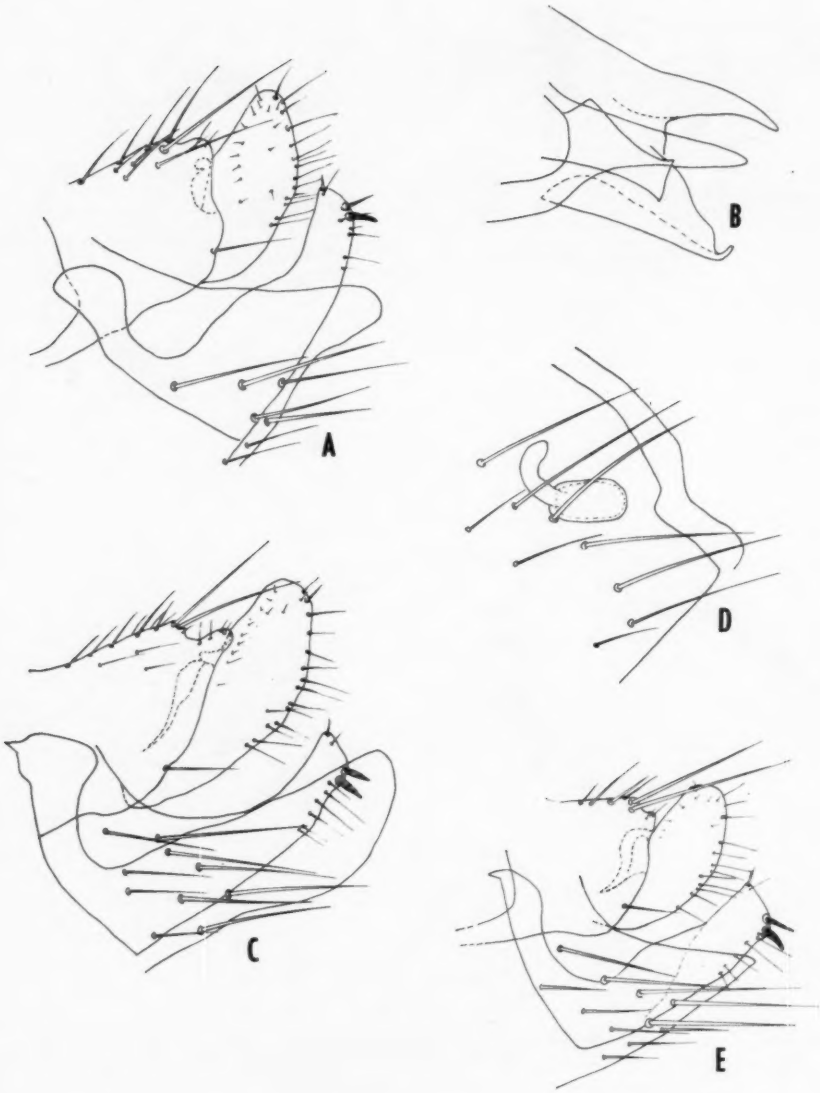


PLATE II



THE LIFE HISTORY OF *SPIRORCHIS ELEGANS* STUNKARD, 1923 (TREMATODA: SPIRORCHIIDAE) FROM THE PAINTED TURTLE*

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Since 1862, when Leared described a blood fluke, *Distomum constrictum*, from the "edible turtle" of England, turtle blood flukes of the family Spirorchiidae have attracted attention by their novelty and by their promise of contributing to knowledge of important flukes of man, the schistosomes.

After Leared's report, sporadic and often incomplete accounts of new species of Spirorchiidae continued for the next 60 years. Stunkard (1923) presented the first scholarly review of the family and described four new species. During the next 12 years workers in America (Stunkard, 1926a, 1926b, 1928; MacCallum, 1926; Price, 1934; and Luhman, 1935), in Europe (Ejsmont, 1927), and in India (Mehra, 1933, 1934; Thapar, 1933; and Sinha, 1934) described new species, reduced others to synonomy, and juggled others between genera, often on the basis of insufficient evidence. Byrd (1939) restored order to a confused situation, and in a review of the family recognized only 8 valid genera of the 17 then extant; he also described 9 new species.

Four life histories have been completed experimentally in the laboratory. The first was that of *Spirorchis parvus* (Stunkard, 1923) from the painted turtle by Wall (1939, 1940, 1941a). Wall (1941b) later determined the cycle of *S. elephantis* (Cort, 1917) also from the painted turtle. In 1951 he described the cycle of *Vasotrema robustum* (Stunkard, 1928) from the soft-shell turtle. The fourth cycle, by Pieper (1953), was that of *S. artericola* (Ward, 1921) also from painted turtles.

The present paper depicts the life history of *Spirorchis elegans* Stunkard, 1923, described originally from two specimens taken from *Pseudemys elegans* Weid from Illinois. Cercariae shed from snails collected at the old Decatur reservoir, DeKalb County, Georgia, were tentatively identified as spirorchiid cercariae; subsequent laboratory studies proved that these larvae penetrated turtles and became adult worms referable to the species *S. elegans*. Painted turtles, *Chrysemys picta picta* (Schneider), also captured at the Decatur reservoir, had adult *S. elegans* in the vascular system and eggs in the tissues. It was these eggs which were used to establish the experimental infections in laboratory raised snails.

MATERIALS AND METHODS

Twenty-six baby painted turtles, *Chrysemys p. marginata* Agassiz, were used in the present investigation. Experimental turtles, average carapace length 34 mm, were obtained from a commercial dealer in Wisconsin (E. G. Steinhilber and Co., Box 888, Oshkosh). Ten turtles from this lot, selected randomly and serving as controls, proved free of any helminth infection after 7 months in the laboratory; in fact, some still had remnants of the yolk sac at this time, indicating that they were recent hatchlings at the time of original receipt.

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Laboratory raised red ramshorn snails, *Helisoma anceps* Menke, served as satisfactory intermediate hosts in the laboratory. Later, a few planorbid snails, *Menetus dilatatus buchanensis* (Lea) collected at the reservoir, were found emitting cercariae identical in form and behavior to those shed by *Helisoma*. Laboratory raised *Menetus* were subsequently successfully infected with miracidia and cercariae were obtained from these hosts in the laboratory.

Eggs were liberated from naturally infected turtles' lungs following the technique advocated by Cable (1950), and were incubated in small stender dishes in pond water changed twice daily. Since most dishes containing incubating eggs were maintained in the laboratory, some variability was observed in time of hatching due to fluctuations in the laboratory temperature. Other containers of embryonated eggs were kept in the refrigerator to facilitate, upon warming, hatching at more convenient times.

Snails (*Helisoma* and *Menetus*) were infected in small containers into which pond water and one to several miracidia were placed. The snail was watched with the stereo-binocular microscope, and if penetration occurred, was subsequently kept in isolation in an individual container. Larval stages within the liver of the snail were removed by crushing the host and transferring the infected organ to a slide for in vivo examination under the compound microscope.

Sixteen turtles were exposed to cercariae in 4-inch finger bowls containing enough aged water to cause the turtle to elevate the head to obtain air. Counted cercariae were transferred by micropipette to the finger bowl; turtles were observed under the microscope during initial phases of cercarial attack and penetration. Areas of attack seemed concentrated on the head and cloacal regions. Five exposed turtles were sacrificed within a week or two of exposure; 5 of the other 11 turtles were killed after 2 months or longer to recover adult flukes. After brief in vivo observations, adult worms were flattened by gentle coverglass pressure and fixed with Gilson's fluid. All whole mounts were stained with Semichon's carmine.

Miracidia were studied alive and unstained in pond water and after impregnation with silver, as described by Goodchild (1948).

Cercariae were studied unstained, and after intravital staining with dilute neutral red and nile blue sulfate. A 0.1 percent solution of polyvinyl alcohol was valuable for quieting cercariae. The P.V.A. was applied by ringing the coverglass and allowing it to diffuse slowly into the pond water preparation. Steaming (60° C.) 10 percent formalin was used to kill miracidia and cercariae for measurements.

We wish to express grateful thanks to Dr. William J. Clench, Museum of Comparative Zoology at Harvard College, Cambridge, Massachusetts, for identifying the two species of gastropods used in this study.

OBSERVATIONS AND RESULTS

1. *Egg*

Eggs were recovered from feces or from macerated host organs, usually lungs. Egg color varied from light amber to reddish brown; the former were unembryonated, whereas the latter, usually fully embryonated, were opaque and internal details were resolved with difficulty. Unhatched, mature eggs (fig. 1) averaged 108 (with a range of 96 to 118) microns in length and 77 (67 to 90) microns in width. The outline was symmetrical and the thin shell was devoid of sculpturing.

The embryo exhibited sluggish activity several days prior to hatching. Epidermal plates and flame cells were visible under oil immersion, but the former showed no ciliary activity until just before hatching. A large gas bubble occupied most of the space between the embryo and one side of the shell. A pair of eyespots was also visible for several days prior to hatching. The operculum was invisible until it began to open; at that time it appeared as a perfectly round lid, measuring less than the diameter of the embryo.

Although eggs from feces have been used successfully as a source of miracidia, eggs from macerated lungs were much more plentiful and for that reason were used. Lung tissue has been stored in the refrigerator in pond water at 6° C up to 76 days with no impairment of miracidial hatching rate or infectivity.

Hatching occurred generally in the early morning hours, usually from 2:00 to 6:00 A.M. Miracidia hatched from eggs in largest numbers in the early morning

hours of the 4th and 5th nights in summer (24° to 32° C), and on the 5th and 6th nights in the fall and winter (16° to 24° C). It was possible to alter hatching time by keeping ripe eggs in the refrigerator until just before miracidia were needed. The container of eggs was placed under a desk lamp and allowed to warm slowly to room temperature. With this technique miracidia were abundant after 45 to 90 minutes and could be used in infection experiments at more convenient times. This modification was a desirable one since miracidia retain infectivity for only about 3 hours.

2. *Miracidium*

The swimming miracidium is elongate oval in shape. The body is widest at the level of the eyespots and tapers gradually to the posterior end, and abruptly to an anterior conical papilla. Cilia cover the body, except on the papilla and the sutures of the epidermal plates. Four specimens fixed in hot formalin had a mean length of 77 (71 to 91) microns, and a mean width of 45 (37 to 48) microns. Epidermal plates are arranged in 4 interlocking tiers with a 6, 6, 4, 2 pattern (fig. 2).

Internal details (fig. 3) are easily visible since living miracidia are transparent. The x-shaped figure formed by the adjoining eyecups is located at the junction of anterior and middle body thirds and lies over a relatively large, rectangular brain. The eyecups appear pyriform with the narrow end directed anteriorly. Each consists of 15 to 20 discrete spherical granules arranged around the periphery.

A pair of massive cephalic glands, with coarse internal contents exhibiting agitated Brownian movement, occupies much of the anterior two-fifths of the body; fine ducts run forward from the glands to open at the denuded apex of the conical papilla. Slightly posterior to the openings of the ducts is an epidermal area with short, compact cilia (fig. 3) which, with the secretions of the glands, may constitute penetrating devices. A so-called primitive gut lies in close association with the cephalic glands and extends posteriorly to the level of the eyes; this structure, whose coarse granular secretory contents may serve an adhesive and penetrating function, was a conspicuous object, but a duct was not seen. Four clusters, in 2 pairs, of extremely fine droplets frequently exuded at the level of the base of the first tier of epidermal plates; no ducts were seen running to these secretions and their function, if not artifactitious, is unknown. An anterior and posterior pair of flame cells are located in the cortical area; ducts run posteriad and anteriad, respectively, from these cells, join, and open laterally slightly behind the anterior edge of the third epidermal tier. Germinal elements, consisting of single large cells and germ balls of 3 or more cells, occupy the posterior central region of the miracidium; nuclei of these cells were large, 7 to 8 microns in diameter, and stained intensely with intravital dyes such as nile blue sulfate.

Within a minute or two before hatching, cilia began beating at an increased rate and caused rapid spinning of the miracidium on its long axis. When the operculum popped open the peri-miracidial vitelline material rushed out followed shortly by the anterior end of the thrusting miracidium. Invariably, the miracidium became wedged, midway, in the opening and freed itself by vigorous contortions. When completely emerged it hovered for a short time, beating its cilia rather slowly and revolving on its long axis. Finally it accelerated away from the empty shell, reached a uniform rapid rate of motion, and swam ceaselessly until it found a suitable host or became exhausted. From time to time it made abrupt changes in direction without obvious cause or slackening of speed. All depths of

the water were occupied and no definite phototaxes were noted.

Behavior of the miracidium near a suitable snail host followed a characteristic pattern which was distinct in a vigorous miracidium, but less faithfully followed as it became exhausted. It would at times swim back and forth beside the snail; as it approached to within about a millimeter, its actions became almost frenzied. It made short circling dashes until contact was achieved; any part of the snail elicited the penetration behavior, even the shell. When contact was made with the head, especially the tentacle, or the foot, the apical papilla adhered and slow oscillations and gentle squirming motions ensued. Almost imperceptibly the miracidium slipped beneath the surface within 1 to 5 minutes. The snail sometimes reacted vigorously to the penetrating attempt, and often succeeded in ridding itself of the attacker, which has never been seen to penetrate successfully at a second attempt; other snails appeared to be unaware of the penetrating miracidium and did nothing in defense. Miracidial invasion of head or foot generally left no mark at the site; invasion of a tentacle, on the other hand, usually produced a marked papular swelling which persisted in some mollusks for as long as 24 hours.

Three young *Menetus*, 1 to 1.5 mm in diameter, died within 24 hours of penetration of each by 1 miracidium. Young snails, in general, were more susceptible to successful penetration, but also succumbed more readily.

Miracidia, at summer laboratory temperatures, were short-lived and were used within 4 hours after hatching; by 7 hours most were dead, and those still living were too weak to penetrate. Refrigerating miracidia at 6° C prolonged their vitality and made infection attempts possible at more convenient hours.

3. *Sporocysts*

The scarcity of infected snails during most of this work greatly restricted investigation of sporocyst development. Larvae, which by position and structure, were believed to be young daughter sporocysts have been found in the liver of *Menetus* 9 days after miracidial penetration. Two sporocysts dissected free of host tissue measured 166 by 61 microns and 141 by 68 microns, respectively. One of these sporocysts was a long ellipse, the other was ovoid with a tapered blunt end; each had a small papilla at one end. The longer sporocyst exhibited feeble motions. Within each was a spherical to oval mass of cells 129 to 132 microns in diameter, considered to be germinal.

Mature daughter sporocysts were long, 800 by 65 microns, and unbranched, with one end embedded in the liver and the other end lying free on the hepatic surface. Sporocysts show constrictions corresponding to intercercarial areas. The wall of the apex was thicker than the lateral walls. Cercarial development was basifugal.

Time for cercarial emission after infection was correlated to temperature. In mid-summer cercariae have emerged as quickly as 15 days after miracidial penetration; in winter the mean emergence time was 20 days. Once cercarial production commenced it may continue for as long as nearly 6 months.

The number of cercariae emitted daily from infected snails was generally small. Forty was a maximum from a young *Helisoma*, 3 to 5 was the usual daily output from *Menetus*. There was variability in output from the same snail; a snail producing 20 cercariae one day might produce 1 or none the succeeding day.

4. *Cercaria*

Spirorchis elegans has an apharyngeate, distomate, furcocercous cercaria (fig.

4). The body has a pronounced fleshy hump (fig. 5), but no true crista. The entire larva is highly mobile, capable of extremes of contraction and extension. Measurements of 10 cercariae from an experimental infection, fixed in steaming 10 percent formalin, are as follows: mean body length 203 (175 to 224) microns, mean body width 64 (49 to 84) microns, tail stem mean length 605 (534 to 658) microns, and mean furcal length 174 (133 to 203) microns. The body is provided with exceedingly short, fine spines. A broad disc of slightly more prominent spines arranged in close order lies around the oral sucker. Tufts of long spines project from each side of the body a little behind the oral sucker; they appear rigid, but are capable of feeble twitches, possibly imparted by the anterior mobility of the body.

The oral sucker is terminal and prominent. A massive, cup-shaped muscular region, the head organ, which extends deeply into the anterior end of the body, contributes extreme mobility to this zone. A pair of small papillae, one on each side of the orifice of the oral sucker, connects directly to the fascicles of ducts from the penetration glands.

The ventral sucker lies one-half to three-fifths of the body length from the anterior extremity. It, too, is capable of extreme projection (fig. 5) and rotation, and is highly muscular; the exposed rim is ornamented with small spines and the inner cup is papillose.

A pair of eyespots is located approximately two-fifths of the body length from the anterior end. They are spherical in shape and are composed of a cluster of dark granules embedded in a clear region lying in, or dorsal to, the brain.

The esophagus is 88 microns long and extends from the oral sucker nearly to the ventral sucker where it joins a pair of short gut ceca. The inner cuticular lining of the esophagus is provided with close-set rings resembling the annular cartilages of the vertebrate trachea.

The genital primordium was resolved with difficulty due to the massive development of the penetration glands in the same region. It is a large mulberry-like mass consisting of about 30 cells, with large, eccentric nuclei. It lies roughly midway between the ventral sucker and the posterior end of the body.

Seven pairs of penetration glands occupy most of the dorsal part of the posterior half of the body. They are arranged in 2 lateral fields, with the swollen portion of each gland dorsal, and the duct directed anteriad and ventrad. Each duct continues as a discrete vessel in the fascicle to the anterior orifice.

Six pairs of flame cells are present in the excretory systems, 5 in the body, and 1 in the anterior tail stem. The flame cell formula is $2[(1+1+1)+(1+1+1)]$. The prominent, Y-shaped excretory bladder leads into a canal which runs the length of the tail, branches at the furcae, and sends a central duct down each branch to the apex where it opens through a small vesicle, at an excretory pore.

The tail stem bears 2 rows of approximately 13 long, regularly spaced spines which project at right angles to the tail stem. Each spine arises singly from a papilla and appears rigid despite the deceptively delicate structure. The furcae depart laterally from the tail stem and each bears a broad, membranous fin projecting dorsally and ventrally, and beyond, each furca. Fins have dense lines, resembling internal supports, projecting diagonally toward the furcal tip.

The delicacy of the cercariae of *S. elegans,* their great speed, and the amplitude of lateral threshing all contribute to making them easily overlooked in water. Their swimming is vigorous and, except for brief periods of crawling on the substrate or

sinking in the water in a tight U-shaped form, continues until they become exhausted and die. They move, usually body forward, rotating on the long axis. The mechanism of tail action is blurred by its great speed and the entire larva, body and stem, has the appearance of a vibrating arc. In motion the body, by optical illusion, appears double, with a bluntly pointed anterior end and the posterior ends divergent at a slight angle. All depths of water are occupied, and numerous tests revealed no response to light.

Cercariae appeared to exhibit chemotaxis toward turtles. When swimming cercariae were added to water in which a small turtle was resting, and the head of the turtle watched with a stereo-binocular microscope, the larvae approached the head and whirled in an agitated fashion. At times they directly attacked the conjunctiva and eyelids and began creeping and thrusting movements. Actual penetration was never observed because the turtle became restless and, if restrained, withdrew the head. Exposures of turtles were made with from 4 to 40 cercariae. More often than not no reactions on the part of the turtle ensued and it is believed that most cercariae failed to attach and penetrate. When turtles manifested irritation it was dramatic. The forefeet clawed and made clumsy wiping motions at the eyes and snout for a few seconds to 15 minutes. On a few occasions, similar awkward clawing movements were made with the hind legs at the cloacal area. A manifestation of irritation which was considered significant was a gaping and snapping movement of the jaws. This was a common response and one which suggests that cercariae enter the nostrils and attack the mucous membranes of the nasal or oral cavities. Cercariae, on occasion, have been observed darting about the nostrils, and once or twice it was believed they entered. Wall (1941a) was of the opinion that cercariae of *Spirorchis parvus* penetrate the skin around the eyes and the cloacal aperture. In *S. elegans* the turtle's behavior suggests attack at these sites plus possible entrance through the nostrils and mucous membrane of the mouth.

The longevity of cercariae of *S. elegans* after emergence was always brief. Cercariae emerging in late evening are nearly all dead or inactive by 8:00 A.M. Larvae, collected in the early morning hours, have been maintained alive, until late afternoon, for morphological studies at 6° C. in the refrigerator; they appeared too feeble to attempt turtle exposures.

5. *Adult*

The adult *S. elegans* (fig. 6) has been adequately described by Stunkard (1923). Details of complete morphology will not be further elaborated upon here. As can be seen in figure 6, however, there appears to be room in the uterus for only a single egg at a time. Stunkard (1923) reported for the same species, "neither specimen contains an egg." This species is hematophagous, and the intestinal ceca are filled with masses of digesting blood. Ulmer (1959) has reported similar food habits for *S. haematobium;* Wall (1941a), on the other hand, reported that adult blood flukes taken from larger arteries and the heart have ceca filled with digesting blood, but flukes from arterioles of the walls of the stomach and intestine were devoid of coloring matter in the ceca.

6. *Experimental Infection of Turtles*

Eleven baby *Chrysemys p. marginata* were exposed to cercariae and maintained in the laboratory for 2 months or longer. Five of these turtles subsequently revealed eggs in the feces and in the tissues post mortem. Five additional turtles yielded worms post mortem, as well as eggs. One of the 11 revealed neither worms nor

eggs when sacrificed. Five additional turtles were exposed in preliminary experiments, but died or were sacrificed prior to 2 months; from these hosts, worms or eggs were not recovered. Adult worms were all referable, as stated previously, to the species *Spirorchis elegans* Stunkard, 1923.

As mentioned above, the 10 control turtles were examined routinely for fecal eggs. No helminth eggs were observed during life and no eggs or worms were found post mortem in the viscera. One control turtle was killed 95 days after the beginning of the experiment, 1 after 100 days, and the remaining 8 after 7 months.

Due to limited numbers of cercariae, turtles were exposed individually in finger bowls. Each turtle was generally re-exposed daily until it responded as though irritated. Seven of 11 turtles were exposed only to cercariae from an experimental *Helisoma* host; 2 others received 6 and 21 cercariae, respectively, from a naturally infected *Menetus*. Two other turtles were exposed only to cercariae emerging from a laboratory infected *Menetus*.

A total of 13 blood flukes was recovered from 5 experimental turtles; these were prepared as stained whole mounts except 1 which was fixed in situ in an auricle. Nine of the 10 turtles, proved subsequently to be infected, were exposed for 10 days or less, and the other for 27 days. The maturation period was arbitrarily taken as the time from the middle of the exposure period to the first appearance of eggs; this interval was from 6 to 10 weeks.

Seven of the 13 worms recovered were found in the heart, 6 in the ventricle and 1 in the auricle; 4 were found in the surface vessels of the brain; one was recovered from the brachiocephalic artery, and another from the liver. Flukes were restricted to the heart or arteries; none was found in the veins, nor in the vessels of the gut wall, a common site reported in other studies by Stunkard (1923), Byrd (1939), Wall (1940, 1941a, 1951), and Pieper (1953).

DISCUSSION

The short time, 6 to 10 weeks, which elapsed between exposure of turtles to cercariae of *S. elegans* and appearance of eggs in feces is an important difference between this cycle and the known cycles of other spirorchiid trematodes. *Vasotrema robustum* of the soft-shell turtle, *Amyda,* required 10 to 12 months to mature and begin releasing eggs from the turtle (Wall, 1951). *Spirorchis parvus* of the painted turtle, *Chrysemys,* required a minimum of 3½ months for the same criterion of maturity (Wall, 1941a). The same author, Wall (1941b), found that *S. elephantis,* also from *Chrysemys,* required a like period of time. Pieper (1953) reported a developmental time of 4 months for *S. artericola* in *Chrysemys*.

Lack of host-organ specificity in the location of the adult worm is a further divergence of *S. elegans* from the typical spirorchiid. Nowhere in the literature has reference been found of the occurrence of adult turtle blood flukes in the brain vessels. In the present study, 4 adult worms were recovered from the meningeal vessels of 2 hosts. One specimen was recovered also from intrahepatic vessels, a new site for adult turtle blood flukes, although Wall (1951) found this to be the customary location of juvenile *Vasotrema robustum*. Livers of additional hosts were routinely inspected without recovering another specimen. Reports of wider distribution of these flukes in their hosts seems assured with additional studies; e.g., Ulmer (1959) has reported *S. haematobium* in the submucosa and peri-connective tissues of the esophagus in painted turtles, *Chrysemys p. belli,* from Iowa. *Spirorchis elegans*

crept forcefully in the lumen of the meningeal arteries. This motion, coupled with the vigorous swimming movements in less confined spaces, suggests that many of the larger and smaller vessels may be natural habitats for spirorchiid flukes.

The consistently small number of flukes in each turtle and the massive accumulation of eggs, particularly in the lungs, suggest that each worm deposits several to many eggs daily. Three to 5 eggs were often observed in a row within the lumen of a blood vessel occupied by a fluke. In no case have miracidia been observed in these eggs. More frequently than not, especially in recently captured turtles, the tissues will be charged with viable, fully embryonated eggs, but no adult flukes can be found. This suggests a relatively short life span of the adults, and indirectly supports the belief in high rates of daily egg output. Although the life span is unknown, living flukes, in an experimental infection, were obtained after 19 weeks of egg discharge by the turtle. Stunkard (1923) recovered living spirorchiids from turtles kept 1 year in the laboratory during which time no opportunity for re-infection had occurred.

Times required for cercariae to develop in the snail host is important as a characteristic of the species, though not always distinctive. Pieper (1953) observed that *Helisoma,* in the summer, began emitting cercariae of *S. artericola* 21 days after miracidial penetration. As temperature varies, the intra-molluscan developmental time of *S. elegans* was altered; only in winter was the initial cercarial shedding time delayed to 3 weeks or longer.

As Cort and Brackett (1937) have suggested, diagnostic information may emerge from recording the swimming characteristics of cercariae. The tendency, as Wall (1941a) reported, of cercariae of *S. parvus* to rest with the body in a loose or tight arc while drifting to the bottom of the container on which it may rest for several minutes differs sharply from the intense, almost ceaseless swimming activities of *S. elegans;* however, *S. elegans* cercariae have been observed to exhibit brief periods of sinking through the water in a reflected position. Cercariae of *S. elephantis* swim with either end forward (Wall, 1941b) and float with the tail stem straight and the furcae variously spread. At rest, the cercaria of *Vasotrema robustum* always has the anterior part of the body and tip of one furca in contact with the bottom (Wall, 1951).

SUMMARY

The life cycle of a turtle blood fluke, *Spirorchis elegans* Stunkard, 1923, has been completed in the laboratory. Young painted turtles, *Chrysemys picta picta* (Schneider), and the planorbid snail, *Menetus dilatatus buchanensis* (Lea), were the natural hosts in DeKalb County, Georgia. *Chrysemys p. marginata* Agassiz was successfully infected with cercariae in the laboratory, as were young *Helisoma anceps* Menke and *Menetus d. buchanensis* with miracidia.

Eggs of the worm, contained in shredded lung tissue, were incubated at room temperature in pond water. Hatching occurred in the predawn hours after 4 nights of incubation (in summer), and after 6 nights (in winter). Miracidia penetrated the exposed surfaces of *Menetus* and the tentacles of *Helisoma.* Daughter sporocysts appeared on the liver within 9 days after exposure of the snail to miracidia.

Furcocercous, apharyngeate cercariae emerged in the late night and early morning hours in 17 to 20 days after exposure of the snail. Cercariae attacked the eye and

cloacal regions and the mucous membranes of the mouth and nostrils of susceptible turtles.

After 6 to 10 weeks, blood fluke eggs were passed in the turtle's feces. Adult flukes were found in the heart, brain, systemic arteries, and liver.

Literature Cited

Byrd, E. E. 1939 Studies on the blood flukes of the family Spirorchidae. Part II. Revision of the family and description of new species. J. Tenn. Acad. Sci. **14:** 116–161.

Cable, R. M. 1950 An Illustrated Laboratory Manual of Parasitology. Burgess Publishing Company, Minneapolis.

Cort, W. W., and Brackett, S. 1937 Identification of strigeid cercariae by differences in their behavior during free life. J. Parasit. **23:** 297–299.

Ejsmont, L. 1927 *Spirhapalum polesianum* n.g., n.sp. trématode du sang d'*Emys orbicularis* L. Ann. Parasit. **5:** 220–235.

Goodchild, C. G. 1948 Additional observations on the bionomics and life history of *Gorgodera amplicava* Looss, 1899 (Trematoda: Gorgoderidae). J. Parasit. **34:** 407–427.

Leared, A. 1862 Description of a new parasite found in the heart of the edible turtle. Quar. J. Micr. Soc. London, n.s. **2:** 168–170.

Luhman, M. 1935 Two new trematodes from the loggerhead turtle (*Caretta caretta*). J. Parasit. **21:** 274–276.

MacCallum, G. A. 1926 Revue du genre Spirorchis MacCallum. Ann. Parasit. **4:** 97–103.

Mehra, H. R. 1933 New blood flukes of the family Spirorchidae Stunkard from Indian fresh-water tortoises with discussion on the systematic position of the genus Coeuritrema n.g. and the relationships of the families of blood flukes. Bull. Acad. Sci. U. P. Agra and Oudh. **2:** 203–222.

——— 1934 New blood flukes of the family Spirorchidae Stunkard from Indian fresh-water tortoises with discussion on the synonymy of certain genera and the relationships of the families of blood flukes. Part II. Bull. Acad. Sci. U. P. Agra and Oudh. **13:** 169–195.

Pieper, M. B. 1953 The life history and germ cell cycle of *Spirorchis artericola* (Ward, 1921). J. Parasit. **39:** 310–325.

Price, E. W. 1934 New genera and species of blood flukes from a marine turtle, with a key to the genera of the family Spirorchidae. J. Wash. Acad. Sci. **24:** 132–141.

Sinha, B. B. 1934. A new genus of blood flukes of the family Spirorchidae from the tortoise, *Hardella thurgi* (Gray). Rec. Indian Mus. **36:** 147–151.

Stunkard, H. W. 1923 Studies on North American blood flukes. Bull. Am. Mus. Nat. Hist. **48** (art. 7): 165–221.

——— 1926a A new trematode, *Vasatrema amydae* n.g., n.sp., from the vascular system of the soft-shelled turtle, *Amyda*. Anat. Rec. **34:** 165.

——— 1926b A new blood fluke, *Unicaecum ruszkowskii* n.g. n.sp; a contribution to the relationship of the blood infesting trematodes. J. Parasit. **12:** 164–165.

——— 1928 Observations nouvelles sur les trématodes sanguicoles du genre Vasotrema (Spirorchidae) avec descriptions des deux espèces nouvelles. Ann. Parasit. **6:** 303–320.

Thapar, G. S. 1933 A new blood fluke from an Indian tortoise, *Trionyx gangeticus*. J. Helm. **11:** 163–168.

Ulmer, M. J. 1959 Studies on *Spirorchis haematobium* (Stunkard, 1922) Price, 1934 (Trematoda: Spirorchiidae) in the definitive host. Trans. Am. Micr. Soc. **78:** 81–89.

Wall, L. D. 1939 Life history of *Spirorchis* sp. J. Parasit. **25:** 28.

——— 1940 Life history of *Spirorchis parvus* (Stunkard) Trematoda: Spirorchiidae. Science, n.s. (No. 2390) **92:** 362–363.

——— 1941a *Spirorchis parvus* (Stunkard): Its life history and development of its excretory system (Trematoda: Spirorchiidae). Trans. Am. Micr. Soc. **60:** 221–260.

——— 1941b Life history of *Spirorchis elephantis* (Cort, 1917), a new blood fluke from *Chrysemys picta*. Am. Midl. Nat. **25:** 402–412.

——— 1951 The life history of *Vasotrema robustum* (Stunkard 1928), Trematoda: Spirorchiidae. Trans. Am. Micr. Soc. **70:** 173–184.

EXPLANATION OF PLATE I

Drawings are based on free hand and camera lucida sketches, and the proportional scale is shown beside each drawing.

FIGURE 1. Mature miracidium within unhatched egg.
FIGURE 2. Miracidial epidermal plate pattern.
FIGURE 3. Free-swimming miracidium.
FIGURE 4. Furcocercous cercaria of *Spirorchis elegans.*
FIGURE 5. Side view of body and anterior tail stem of cercaria of *Spirorchis elegans.*
FIGURE 6. Adult *Spirorchis elegans* from *Chrysemys p. picta;* natural infection, DeKalb County, Georgia.

RESEARCH NOTE

THE INFECTIVITY OF FOUR GEOGRAPHIC STRAINS OF *SCHISTOSOMA JAPONICUM* IN THE RHESUS MONKEY.

Since the Formosan strain of *Schistosoma japonicum* has been proved to be a non-human strain (Hsü and Hsü, Am. J. Trop. Med. Hyg. **5**: 521–528), further study concerning the resistance to this strain in man seems desirable. As it is difficult to obtain human volunteers for such a study, it is hoped that one of the common simian animals can be used as a substitute. It has been demonstrated that the Formosan monkey *Macaca cyclopis* was not susceptible to the infection of the Formosan strain of *S. japonicum,* although the Japanese monkey *M. fuscata* and the Philippine monkey *M. philippinensis* made reasonably good hosts for this strain (Hsü and Hsü, 1956, Am. J. Trop. Med. Hyg. **5**: 136–144). A difficulty with the Formosan monkey is that it is not easily procured outside Formosa. Since the rhesus monkey is now a common laboratory animal which can be obtained from India in large numbers, it was of interest to ascertain the infectivity of the various geographic strains of *S. japonicum* in this animal, especially of the Formosan strain.

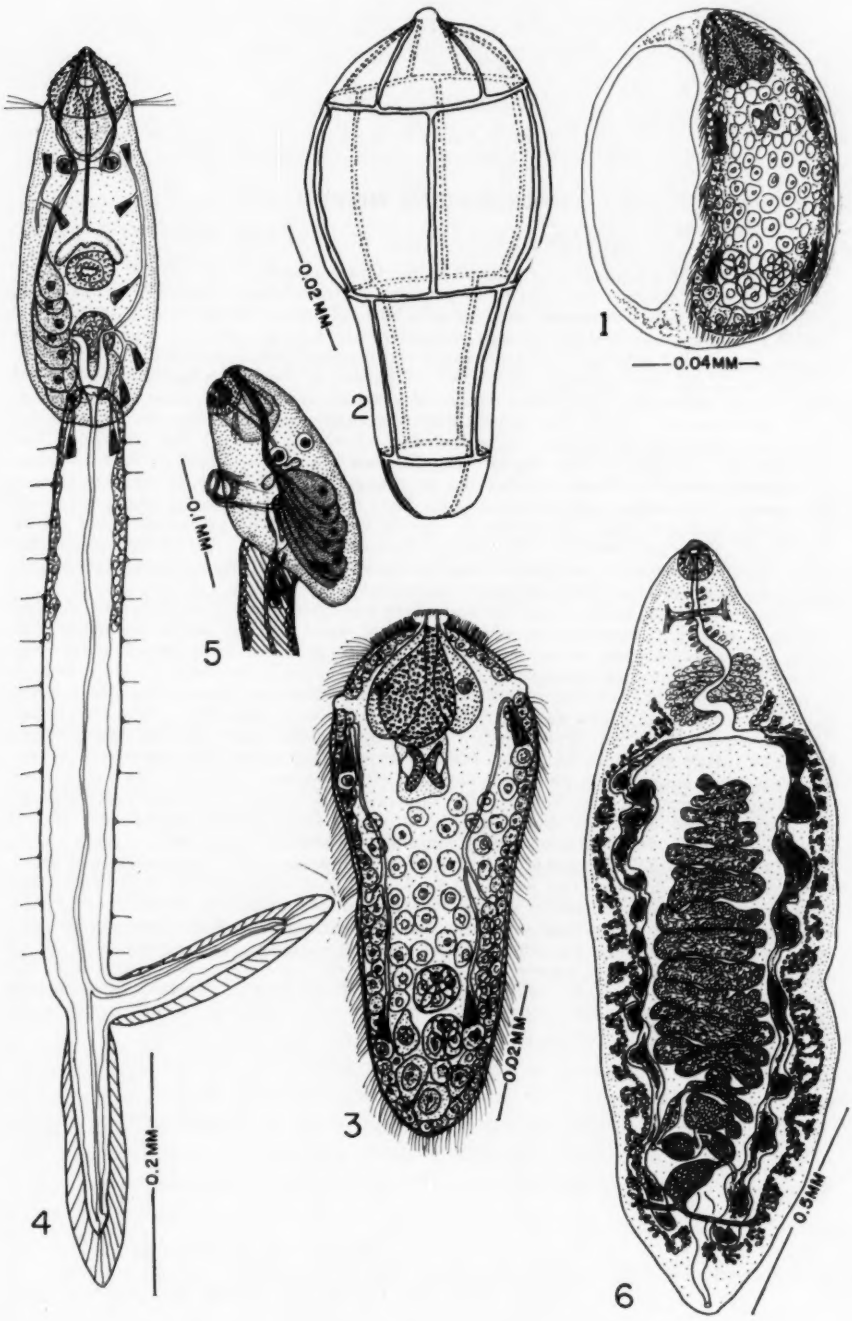
Four geographic strains of *S. japonicum* were used: The Chinese, Formosan, Japanese, and Philippine. The cercariae of the Chinese strain were obtained from *Oncomelania hupensis,* originating in Kashing, Chekiang, China; the Formosan, from *O. formosana* obtained from Changhua, Taiwan, China; the Japanese, from *O. nosophora* obtained from Kofu, Japan; and the Philippine, from *O. quadrasi* obtained from Leyte, the Philippines. All the rhesus monkeys were purchased from the Shamrock Farms, New York, and were identified by us as Indian rhesus, *Macaca mulatta.* They were about 4 pounds in weight and before the infection proved to be negative for schistosome eggs in the stools. Each monkey was infected by the cutaneous route, using 400 cercariae with the sexes balanced. The cercariae were shed from infected snails. Seven monkeys were used. One was infected with the Chinese strain, 1 with the Japanese strain, 1 with the Philippine strain, and 4 with the Formosan strain. Beginning from the 30th day of infection, the stools of each monkey were examined daily. The eggs were concentrated in physiological saline by the sedimentation method.

In the monkeys infected with the Chinese, Japanese, and Philippine strains, schistosome eggs were found in the stools, respectively, on the 39th, 33rd, and 35th day of the infection. The 4 monkeys infected with the Formosan strain, however, were negative for the schistosome eggs in the stools throughout 70 days of daily examinations. On the same date when the 4 monkeys were infected with the Formosan strain, 200 cercariae of the same strain were used to infect 2 albino mice. The 2 mice were positive for the schistosome eggs on the 42nd day of the infection.

It was concluded that the rhesus monkey, like man and the Formosan monkey, is not susceptible to infection with the Formosan strain of *S. japonicum,* although it is susceptible to infection with the Chinese, Japanese, and Philippine strains of this parasite.

This investigation was supported by a research grant E-939 (C4), P.T.M. (5), from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, United States Public Health Service, and by a research grant from the Abbott Laboratories, North Chicago, Illinois.—H. F. HSÜ AND S. Y. LI HSÜ, *Department of Hygiene and Preventive Medicine, State University of Iowa, Iowa City, Iowa.*

PLATE I



RESEARCH NOTE

HUMAN SPARGANOSIS IN ALABAMA

In March 1959 a helminth was referred to the Communicable Disease Center for identification by the Alabama State Health Department. Examination revealed a milky-white, pseudo-segmented worm measuring approximately 94 by 4 mm. The club-shaped anterior end showed a shallow groove and the posterior end was rather thin and irregular. In all significant respects it resembled published descriptions of larval pseudophyllidean cestodes.

The larva had been submitted by Dr. A. J. Treherne of Atmore, Alabama. He reported that a woman patient had come to him because of a nodule near the right breast. After examination, he advised her to wait for a time to see if it might disappear. She returned within 3 weeks with complaints of intense itching and discomfort, and reported that the nodule had migrated about 2 inches from the original site. Thinking it was an inflamed, pustulated abscess, the physician lanced the nodule and observed the emergency of a ribbon-like organism about 5 inches long. The specimen was submitted to the State Health Department for identification and subsequently was sent here. Unfortunately, specific identity of this sparganum could not be determined. Faust and Russell (1957, Clinical Parasitology, 6th ed.) are of the opinion that most or possibly all cases of non-proliferating sparganosis reported from this area of the United States may be classified as *Diphyllobothrium mansonoides*.

Efforts were made to determine how the patient may have acquired the infection. Atmore is a small, semi-rural community in which the patient stated she had resided for the past 20 years. They use city water, do not travel widely, and do not eat exotic or unusual foods. She and her husband go fishing occasionally, but stay at commercial fishing lodges. Thus, there is no real indication as to how the patient became infected. However, it seems possible that she may at some time have gotten water from a shallow well which contained an infected copepod.

This appears to be the fourth reported case of sparganosis in man in the United States. Stiles (1908, Hyg. Lab. Bull. No. 40, pp. 7–18) first reported a case of infection with a pseudophyllidean larva from Florida which was identified as *Spirometra proliferum*. Two subsequent cases have been reported from Texas, one by Moore (1914, Am. J. Trop. Dis. Prev. Med. **2:** 518–525), identified as *S. mansonoides*, and the second by Read (1952, J. Parasit. **38:** 29–31), which could not be identified as to species. In addition, Mueller and Coulston (1941, Am. J. Trop. Med. **21:** 399–425) were able to establish larval infection with *S. mansonoides* in themselves as well as in monkeys, rats, mice, and the leopard frog. The latter workers believe that human sparganosis may well extend throughout the wide species range in which the adult cestodes are found. The author is in agreement with their suggestion that all "fatty tumors" and "cysts" removed from under the skin should be carefully examined for the presence of larval worms. The detection of the present case was due entirely to the alertness and interest of the physician.—NEVA N. GLEASON, *United States Public Health Service, Communicable Disease Center, Atlanta, Georgia.*

STUDIES ON SCHISTOSOMIASIS. XIII. SCHISTOSOME DERMATITIS IN COLORADO*

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Schistosome dermatitis, variously designated "swimmer's itch," "Cercarien-dermatitis," "sawah itch" and "koganbyo," is widespread throughout the world (Chu, 1958). Cort (1928a, b) at Douglas Lake, Michigan, demonstrated that the cercariae of certain species of bird and mammal schistosomes could penetrate human skin and produce a characteristic dermatitis. Schistosome dermatitis has since been reported from many fresh-water areas in the United States and Canada (Cort, 1950; Miller and Munroe, 1951; Chu, 1958). More recently, dermatitis-producing cercariae have been found in brackish and salt water (Penner, 1942, 1950, 1953; Chu, 1952; Leigh, 1953, 1955; Grodhaus and Keh, 1958).

Persons bathing in Grant Lake (at an elevation of approximately 9000 feet) in the summer of 1956 experienced dermatitis. The circumstances surrounding this outbreak suggested that it may have been caused by schistosomes. Therefore, during the summer of 1957 an investigation was made and the history of the 1956 cases of dermatitis reviewed. As a result, it was decided that schistosome dermatitis-producing cercariae were the probable cause, although it was not possible to prove this experimentally.

DISCUSSION OF FINDINGS

Snail Infections Found and Their Significance

Both *Physa propinqua* Tryon and *Helisoma trivolvis plexatus* Ingersol were present in the lake. (Appreciation is expressed to Dr. William J. Clench, Museum of Comparative Zoology, Harvard University, for identifying these hosts.) The former was more abundant and was found along the shore on rocks, wood, or the undersurface of the pond lily leaves (*Nuphar polysepalum*).

A total of 654 snails was collected from Grant Lake in the summer of 1957 and held for a 48-hour period to check for cercarial shedding. Of this total, 547 were *P. propinqua* and 107 *H. trivolvis plexatus*. The following cercarial groups were encountered from *P. propinqua:* schistosomes, 7 (1.3 percent); strigeids, 25 (4.6 percent); monostomes, 40 (7.3 percent); echinostomes, 99 (18.1 percent). Twenty-eight of the 107 *H. trivolvis plexatus,* or 26.2 percent, were infected with echinostomes. Because of the short time available for this study, it was not possible to carry the identification of cercariae further except in the case of the schistosome dermatitis-producing cercariae which were found in 7 of the *Physa propinqua* examined. These cercariae were identified as *Trichobilharzia physellae* (Talbot, 1936) on the basis of the following characteristics: behavioral patterns, responses to light, time of emergence, flame cell pattern, and the detailed morphology of both living and preserved specimens.

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Schistosome Dermatitis Experiments on Volunteers

Cercariae of *T. physellae* were harvested in early August, transferred in aliquots of 25 to the flexor surface of the forearms of 6 volunteers. The drop containing the cercariae was smeared over the surface to avoid runoff, the area was marked with a pen, and allowed to air dry for 30 minutes. Plain water controls in which uninfected snails had been living were placed upon the other forearm. The results are summarized in table I.

TABLE I. *Summary of responses to aliquots of 25 cercariae of* Trichobilharzia physellae *in human volunteers.*

Case No.	Sex	Age group	Primary prickling	Appearance of macules in hours	Reactions in 24 hours			Subsequent history
					None	Macules	Papules	
1(GWH)	M	50–59	+	5		5	0	None in 14 days
2(CR)	M	30–39	–	—	–	0	10	None in 14 days
3(SAAE)	M	20–29	–	4		5	5	*
4(AWH)	F	50–59	–	14	–	0	0	None in 14 days
5(Gay)	F	10–19	+	8		8	0	None in 14 days
6(Jay)	M	10–19	+	4		4	0	Slight pruritus during 24 hours. No other response in 14 days

* Five macules persisted 48 hours, faded, followed by 5 papules and erythema beginning in 24 hours and persisting for 5–6 days with intermittent pruritus.

Five of the 6 volunteers who submitted to experimental exposure in 1957 to the cercariae of *T. physellae* gave minimal responses. Their reactions would indicate that they had not developed a hypersensitivity to this species. This is rather interesting since one of these five (GWH) had been exposed previously and had developed papules and pruritus in the experiments at Green Lake, Seattle, in 1947 (Hunter et al 1949). This raises some interesting speculations on the duration of such sensitivity. The sixth volunteer (No. 3 in table I) gave evidence of a moderate hypersensitivity.

Probable Life Cycle of the Schistosomes Found

Inquiries revealed that usually two or more families of mallard ducks are raised on the lake each year with the peak of the egg-laying occurring in June (Parmalee, 1958). Young birds were encountered early in July. It appears probable that the adults were infected when they arrived in the spring. This species is suspected of being the host, since *T. physellae* (which was found in Grant Lake snails) is known to mature in mallards. However, a single bird examined in 1958 was negative.

Brewer's and red-winged blackbirds were also present and must be considered as possible hosts even though this parasite has not been reported from these birds.

Epidemiologic Considerations

The outbreaks of dermatitis among bathers in Grant Lake were sporadic. Cases were reported in 1956, and 7 cases were seen in 1958, although none was encountered in 1957. This is interesting, since schistosome-bearing snails were recovered in August, 1957. However, not much swimming was done during the summer of 1957, presumably because of the colder weather and rain, which may account for the absence of cases that year.

In 1958, 4 of the 7 cases of schistosome dermatitis seen among bathers from Grant Lake in mid-August gave a typical hypersensitive reaction showing large papules with vesicle formation. All experienced bouts of pruritus and 2 were edematous.

Grant Lake is a small, spring-fed lake, roughly round and approximately one-fourth mile in diameter. Willows emerge from the shallow water at the north end, while much of the south half of the lake is filled with pond lilies (*Nuphar polysepalum*). The swimming area is situated on the northeast side of the lake. Here the shore drops off fairly abruptly and there is open water. The willows at the north end of the lake support only *Physa propinqua*. However, the ducks feed and swim among the pond lilies and other vegetation in other parts of the lake where this snail is abundant. The history of dermatitis apparently followed periods of swimming when the wind blew from the west and northwest across the lake from the area where infected snails had been found.

Among the 547 *P. propinqua* examined, cercariae of *T. physellae* were found in 7 collected near the swimming area along the north side near the willows and halfway down the west side of the lake. Snails of this species from other areas were negative for schistosome cercariae. The location of the positive snails and the sporadic occurrence of the dermatitis suggests a relationship between the location of the infected snails, the direction of the wind, and the swimming area.

If one assumes that the bird host was infected upon its arrival, the relatively cold water of this spring-fed lake (17 to 20° C) would probably have retarded the development of cercariae in the snail host. This in turn might explain why cases of dermatitis did not occur until mid-August.

So far as is known, this is the first record of the existence of schistosome dermatitis in Colorado. Undoubtedly more cases will be found when suspect areas are studied, or when physicians become alerted to the situation.

SUMMARY

Reports received in the summer of 1957 suggested an outbreak the previous year of schistosome dermatitis among bathers at an elevation of about 9000 feet. An examination of snails yielded brevifurcate cercariae from seven specimens of *Physa propinqua* Tryon. These parasites were identified as *Trichobilharzia physellae* (Talbot, 1936), a bird schistosome, the adults of which are known to occur in mallard ducks. Exposure of 6 human volunteers yielded minimal responses in 5 persons and a hypersensitive reaction in 1, indicating that the cercariae penetrated the skin in these individuals.

This is believed to be the first record of schistosome dermatitis from Colorado.

REFERENCES

CHU, G. W. T. C. 1952 First report of the presence of a dermatitis-producing marine larval schistosome in Hawaii. Science **115:** 151–153.

———1958 Pacific area distribution of fresh-water and marine cercarial dermatitis. Pac. Sci. **12:** 299–312.

CORT, W. W. 1928a Schistosome dermatitis in the United States (Michigan). J. Am. Med. As. **90:** 1027–1029.

———1928b Further observations on schistosome dermatitis in the United States (Michigan). Science **68:** 388.

———1950 Studies on schistosome dermatitis. XI. Status of knowledge after more than twenty years. Am. J. Hyg. **52:** 251–307.

GRODHAUS, G. AND KEH, B. 1958 The marine dermatitis-producing cercariae of *Austrobilharzia variglandis* in California (Trematoda: Schistosomatidae). J. Parasit. **44:** 633–638.

HUNTER, G. W., III, SHILLAM, D. S., TROTT, O. T. AND HOWELL, E. V., JR. 1949 Schistosome dermatitis in Seattle, Washington. J. Parasit. **35:** 250–254.

LEIGH, W. H. 1953 *Cercaria huttoni*, sp. nov., a dermatitis-producing schistosome larva from the marine snail, *Haminoea antillarum guadalupensis* Sowerby. J. Parasit. **39:** 625–629.

———1955 The morphology of *Gigantobilharzia huttoni* (Leigh 1953) an avian schistosome with marine dermatitis-producing larvae. J. Parasit. **41:** 1–8.

MILLER, M. J. AND MUNROE, E. 1951 Schistosome dermatitis in Quebec. Can. Med. As. J. **65:** 571–575.

PARMALEE, D. 1958 Personal communication.

PENNER, L. R. 1942 Studies on dermatitis-producing schistosomes in eastern Massachusetts, with emphasis on the status of *Schistosomatium patholcopticum* Tanabe, 1923. J. Parasit. **28:** 103–116.

———1950 *Cercaria littorinalinae* sp. nov., a dermatitis-producing schistosome larva from the marine snail, *Littorina planaxis* Phillipi. J. Parasit. **36:** 466–472.

———1953 The biology of a marine dermatitis-producing schistosome cercaria from *Batillaria minima* (Gmelin). J. Parasit. **39:** 19–20.

RESEARCH NOTE

DIETHYLCARBAMAZINE THERAPY IN BOVINE CYSTICERCOSIS.

As a sequel to the successful application of diethylcarbamazine in human patients with subcutaneous cysticercosis reported by Mazzoti and Treviño (1957, Ann. Rev. Med. **8:** 423) and through the generosity of Lederle Laboratories Division, a small experiment was carried out to evaluate the larvacidal effect of this drug in *C. bovis* infections in cattle.

Eight steers aged 2 to 3 years were randomly selected from a pool of cattle in which the incidence of naturally acquired cysticercosis approached 70 percent. Daily doses of 10, 20, 20, and 40 mg of diethylcarbamazine per kg body weight in the form of "Hetrazan" tablets were administered orally for 7 to 10 days to 4 of the steers; on a weight-for-weight basis these doses were 1.5 to 6 times the recommended dose for onchocerciasis in humans. Toxic signs were not observed in any of the cattle which were maintained at grass until slaughtered 8 to 14 weeks later.

TABLE I. *Evaluation of larvacidal effect of diethylcarbamazine in* C. bovis *infection in cattle.*

Steer number	Daily dose per kg body wt. (mg)	Total dose given (gm)	Interval between treatment and autopsy (weeks)	Cysticerci present	
				Living	Dead
1	10	22.5	8	0	0
2	20	50	8	7	2
3	20	50	8	29	2
4	40	70	14	4	2
Control 1	—	—	—	54	3
Control 2	—	—	—	8	1
Control 3	—	—	—	11	1
Control 4	—	—	—	0	0

At autopsy all except the animal given the lowest dosage level and one of the controls had living and dead cysticerci (table I). A cysticercus was considered to be "living" when the cyst and contents appeared macroscopically normal and "dead" when the cyst was filled with necrotic material or granular pus. The presence of a proportion of dead cysticerci was not considered significant because these are common in such cattle. Similarly the possibility of reinfection with fresh cysticerci between dosing and slaughter was considered unlikely because we have consistently failed to infect East African steers of this age with *Taenia* eggs proven viable in young calves. Resistance to reinfection in adult cattle is extremely widespread and is due to immunity acquired from infection in early life (Urquhart, in preparation). Diethylcarbamazine in the dose-range described above does, therefore, not appear to be of value in the treatment of *C. bovis* infection in cattle.—G. M. URQUHART, *East African Veterinary Research Organization, P. O. Box 32, Kikuyu, Kenya.*

ON THE LIFE HISTORY OF *PROSTHODENDRIUM* (*ACANTHATRIUM*) *ANAPLOCAMI* N. SP. (TREMATODA: LECITHODENDRIIDAE)*

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In a survey of larval trematodes infecting snails in the vicinity of Mountain Lake Biological Station, Mountain Lake, Virginia, 16 species of cercariae were encountered, some of which are probably not yet described. Among them, one minute virgulate xiphidiocercaria was found experimentally to be the larva of a lecithodendriid species belonging to the subgenus *Acanthatrium*. As adult worms reared experimentally in laboratory mice could not be allocated to any reported species, they are described here as *Prosthodendrium* (*Acanthatrium*) *anaplocami* n. sp.

The genus *Acanthatrium* Faust, 1919, was reviewed by Cheng (1957), who gave a key to 14 species, including two new ones. Koga (1954) named and described some life history stages of a fifteenth species, *Acanthatrium hitaense*, a possible synonym of *A. ovatum* Yamaguti, 1939, according to Yamaguti (1958). Skarbilovich (1948) divided the genus *Acanthatrium* into two subgenera, *Acanthatrium* Skarbilovich, 1947, and *Mesothatrium* Skarbilovich, 1947, the latter containing one species, *Acanthatrium japonicum* (Yamaguti, 1939). The apparently erroneous date, 1947, appended by Skarbilovich to these subgenera is unexplained, since she has no recorded 1947 publication dealing with this topic. Sogandares-Bernal (1956) elevated *Mesothatrium* to generic level, but Cheng (1957) recommended that this name be suppressed as either genus or subgenus. According to Yamaguti (1958), Dubois (1955) revised several lecithodendriid genera, suppressing *Acanthatrium* as a genus, but retained the subgenus *Acanthatrium* in the genus *Prosthodendrium* Dollfus, 1931. Dubois also transferred *Lecithodendrium chilostomum* (Mehlis, 1831) and *L. pyramidum* (Looss, 1896) to *Prosthodendrium*. In accordance with Dubois' revision, Yamaguti (1958) placed all described species of *Acanthatrium*, except *A. amphidymum* Cheng, 1957, and *A. oligacanthum* Cheng, 1957, in *Prosthodendrium* Dollfus, 1931, subgenus *Acanthatrium* Skarbilovich, 1948 (note correction in last date).

MATERIALS AND METHODS

A minute species of virgulate xiphidiocercaria was shed by 3 of 1,092 isolated specimens of *Anaplocamus dilatatus* (Conrad) collected in Sinking Creek, near Mountain Lake Biological Station, in the summers of 1956 and 1957 and the spring of 1957. Crushing of 181 snails revealed one immature infection, which was used for study of the sporocysts and developing cercariae. For morphological studies, living, newly emerged cercariae were stained with neutral red and chilled in a refrigerator for about an hour. This treatment made the excretory system more easily observed and slowed degenerative processes in these larvae. One of the infected snails lived for 48 days in an aerated aquarium, but the others died in 4 and 12 days, respectively.

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Cercarial penetration studies were confined to local aquatic larval insects maintained in an aërated container. Only nymphs of the mayfly, *Hexagenia bilineata* (Say), were entered by cercariae, usually through the abdominal gill integument. As many as 70 identifiable cercarial bodies were found moving about in the hemocoel and muscle tissue of the nymphs within 1 hour of penetration. Large metacercariae, later identified as this species, were found unencysted in nymphs from the places where infected snails were found. As nymphs collected in a small branch of Stony Creek, Va., were found to be free of these worms, they were used as the source of metacercariae used to infect mice experimentally.

Since metacercariae did not encyst after 28 days following cercarial penetration, entire nymphs were fed to 8 white mice, which had been starved for 24 to 36 hours. The mice ate them readily, but yielded no flukes when autopsied at intervals of 12 hours. Mice were then temporarily abandoned as unsuitable hosts and day-old chicks were substituted. Using mayflies that had been exposed to cercariae as many as 51 days previously, 12 experiments with chicks proved to be negative. At this time, some of the largest mayfly nymphs molted and emerged as subimagoes and adults. Eight were fed to 3 mice, and several juvenile flukes were found in each when autopsied after 5, 7, and 12 days, respectively. Dissection of 6 newly emerged mayflies subsequently revealed that encystment of the metacercariae had recently occurred. With 9 additional mice, it was determined by feeding them experimentally infected insects that adult flukes would develop if post-nymphal mayflies were used, but not when nymphs were employed. This peculiarity is probably the basis for the great variation observed in the size of metacercarial cysts; the worms apparently encyst, regardless of size, at the time of the subimaginal molt of the host. This phenomenon may also explain the great variation in body size among experimentally produced adults, as well as in natural acanthatriid populations observed by the writer in bats from Arkansas, Kentucky, Illinois, Indiana, and Virginia.

Specimens were fixed in warm Bouin's fluid, stained with Semichon's acetic acid carmine, and mounted in gum damar.

LIFE HISTORY OF *Prosthodendrium anaplocami* N. SP.

Adult (Fig. B)

Based on whole mounts and in vivo studies of 19 worms from the small intestine of experimentally infected mice; all measurements below given in millimeters.

Body ovoid to pyriform, 0.130 to 0.320 long, 0.082 to 0.178 wide. Cuticular spines very small, scattered, extending almost to posterior end of body. Oral sucker antero-ventral, 0.035 to 0.062 in diameter. Prepharynx very short, often apparently absent; up to 0.004 long. Pharynx ovoid to subspherical; 0.012 to 0.021 long, 0.014 to 0.021 wide. Esophagus characteristically looped, 0.009 to 0.019 long. Ceca heavy walled, extending laterally toward testes, 0.024 to 0.038 long, 0.009 to 0.024 in greatest diameter. Acetabulum just posterior to midbody, between testes, often thrust forward and elevated; 0.026 to 0.042 in diameter. Testes subspherical, 0.027 to 0.042 in diameter, with vasa efferentia arising on antero-medial surfaces. Vas deferens very short, forming just at point of entry to prostatic mass. Seminal vesicle and ejaculatory duct coiled within prostate cell mass, 0.012 to 0.025 wide, 0.025 to 0.036 long, lying transversely between gut bifurcation and acetabulum. Wall of genital atrium muscular, armed with numerous slender spines, 0.005 to 0.016 long, directed toward genital pore at antero-sinistral margin of acetabulum. Ovary smooth, ovoid, intertesticular behind acetabulum, usually dextral, 0.022 to 0.037 long, 0.018 to 0.029 wide. Oviduct short, passing postero-medially to enter ootype. Vitellaria dorsal in antero-lateral fields which may meet in mid-line; small follicles arranged in branching cords; primary vitelline ducts pass posteromedially, joining to form vitelline reservoir just anterior to ootype lying just behind acetabulum. Seminal receptacle of uterine type, small, sinistral to ootype. Uterus thin-walled, its coils filling posterior third of body, terminating on left wall of genital atrium. Eggs operculated, unembryonated; 0.018 to 0.023 long, 0.009 to 0.014 wide. Excretory bladder thin-walled, bifurcated, often filling posterior third of body. Excretory duct short, with conspicuous sphincter, its pore in shallow depression at posterior end of body. Excretory tubules and flame cells of the Mesostoma type with the formula $2\,[(2+2+3)+(2+2+2)]$.

Holotype specimen: U.S. National Museum, Helm. Collection No. 39015.

Cercaria (Fig. A)

Description based on living, newly emerged specimens, stained intra-vitally.

Minute, virgulate xiphidiocercariae. Body ovoid to sub-hemispherical with concave ventral side when swimming, 0.044 to 0.081 long, 0.023 to 0.046 wide; cuticular spines inconspicuous, sparsely scattered over entire body; tail cylindrical, tapering, lacking either spines or setae; inserted on body in posterior socket, often appearing coiled when contracted; 0.048 to 0.085 long,

0.007 to 0.018 wide at base. Oral sucker well developed, 0.017 to 0.035 in diameter. Stylet relatively large, with lateral and dorsal protuberances, ventrally curved point; 0.016 to 0.019 long, 0.003 to 0.005 wide at shoulder. Virgula organ slender, small, lying along posterior curve of oral sucker, 0.010 to 0.016 wide, 0.002 to 0.004 in diameter. Prepharynx up to 0.003 long. Pharynx ovoid to subspherical, 0.004 to 0.009 long, 0.005 to 0.007 wide. Esophagus slender, straight, 0.006 to 0.009 long. Ceca poorly differentiated, 0.008 to 0.014 long, 0.003 to 0.007 in greatest diameter. Acetabulum poorly differentiated, often only a muscular bulge just anterior to excretory bladder; 0.015 to 0.019 in diameter. Three pair of cephalic glands dorsal to acetabulum; 0.006 to 0.011 in transverse diameter; their ducts forming a single bundle on each side pass anteriorly, dorsal to oral sucker to open on its dorso-lateral wall on each side of stylet. Two pairs of glandular cells with large granules lying between oral sucker and cephalic glands on each side, no ducts serving them; 0.002 to 0.004 in diameter. Genital primordium a triangular mass just along anterior edge of acetabulum. Excretory bladder small, V-shaped, thin walled; excretory duct short, leading to small pore situated within tail socket on ventral side. Flame cells and excretory ducts of the Mesostoma type with the formula 2 $[(2+2+2)+(2+2)]$.

Cercariae emerge in large numbers daily; they swim vigorously and continuously until exhausted and die within 11 hours at room temperature. They exhibit no phototaxis or periodicity of emergence in the laboratory. Water currents stimulate more energetic swimming motion. Penetration of mayfly nymphs results in decaudation and depletion of cephalic gland contents, but this occurs also in spent cercariae which have not penetrated an insect.

Metacercaria (Fig. D)

Description based on living specimens dissected from muscle tissue of experimentally infected *Hexagenia bilineata* nymphs. (Although unencysted, metacercariae as large as they grow in this host.) Body ovoid to pyriform, sluggish, 0.070 to 0.300 long, 0.040 to 0.078 wide; with small, scattered cuticular spines. Oral sucker subterminal, 0.021 to 0.036 in diameter; frequently bearing a remnant of stylet. Acetabulum prominent, anteriorly directed, protruding; 0.015 to 0.024 in diameter. Pharynx ovoid, 0.005 to 0.009 long, 0.005 to 0.008 wide. Prepharynx 0.005 to 0.008 long. Esophagus straight, slender, 0.005 to 0.009 long. Ceca not well differentiated, 0.012 to 0.015 long, extending laterally toward anterior edges of testes. Three pair of ellipsoidal bodies lying just posterior to ceca; 0.003 by 0.010; contents granular, dark staining; lacking ducts. Primordia of testes, prostatic mass, and ovary small, widely separated, situated as in adult. Vitelline follicles in antero-lateral fields, dorsally situated. Excretory bladder V-shaped, frequently expanded to fill most of posterior third of body. Flame cells and ducts obscured, incompletely observed; of the Mesostoma type with more than 8 flame cells on each side. Numerous small cystogenous glands on the dorsal side.

Encysted metacercariae occur in the thoracic and abdominal muscles of subimago and adult mayflies, and are 0.030 to 0.070 in diameter. The cyst membrane is thin, transparent and flexible. "Precystic" metacercariae are uninfective to mice and chickens.

Sporocyst (Fig. C)

Descriptions based on hundreds of living and permanently mounted specimens from three snails. (One contained over 700 sporocysts in the hepatopancreas and gonads.) Colorless, ovoid to elongate sacs separating readily from host tissues; 0.180 to 0.950 long, 0.095 to 0.350 wide. Birthpore terminal with prominent sphincter, often protruded as a peduncle. Body wall relatively thicker in small specimens. Germ balls, developing cercariae, and completely formed cercariae present; no immature cercariae seen free in host tissues. Slight motility of sporocysts noted. Number of cercariae increasing with size of sporocyst, from 4 (in 1 small sporocyst) to an estimated 150 in a large specimen.

DISCUSSION

Prosthodendrium (*Acanthatrium*) *anaplocami* is distinguished from most acanthatriid species by its small size, probably due in part to the short period of growth (5 to 12 days) allowed, as shown in the experiments described above. Variation in atrial spine length is also greater than seen in described species.

Cuticular spines, present in *P. anaplocami* on the entire body, are found only on the anterior third of *P. molossidis* Martin, 1934, and are absent in all other species. The diagnosis of *Acanthatrium oligacanthum* given by Cheng (1957) states that the body is spinous, but his figure and table of comparison indicate that spines are lacking in this form. Smaller egg size differentiates *P. anaplocami* from *P. eptesici* Alicata, 1932, and *P. alicati* Macy, 1940. The form, position, and spine arrangement of the genital atrium in the present species further differentiates it from the other members of the subgenus *Acanthatrium*.

The life cycle demonstrated here for *Prosthodendrium anaplocami* is similar to that shown by Brown (1926) for the bat fluke, *Prosthodendrium chilostomum*. Brown noted that metacercariae develop unencysted in caddis fly larvae, and that encystment occurred when the pupation of the host took place. The present species has a similar delay of encystment in mayfly nymphs, indicating that cyst formation in such species may be inhibited by the host until just prior to its emergence from the aquatic phase of the life history. Other studies, notably those of Koga (1954) on *P. hitaense* developing in stone fly larvae, Abdel-Azim (1936) on *P. pyramidum* developing in anopheline larvae, and Seitner (1945) on *Cercaria nyxetica* developing in mayfly nymphs, show that encystment is not always delayed so long, occurring after 2 days in *C. nyxetica*. The cercaria of *P. anaplocami* is easily distinguished from larvae of the above 3 species, and from the 4 virgulate xiphidiocercariae described by Seitner (1945), by its minute size, lack of spines or setae on the tail, and the size and position of cephalic glands.

Previous studies noted above indicate that the validity of the genus *Acanthatrium* Faust, 1919, is doubtful. Personal studies of hundreds of adult flukes belonging to the acanthatriid-prosthodendriid complex, taken from bats collected in Arkansas, Kentucky, Illinois, Indiana, Ohio, and Virginia, have led to the conviction that they are congeneric in every respect, except in respect to the spined genital atrium. When such a prominent structure exists in such an otherwise homogeneous group, it is tempting to regard it as generic in value, as Cheng (1957) and others have done. It should be pointed out that the species of *Acanthatrium* form a graded series, ranging from *A. oligacanthum* with only 9 atrial spines, 2.6 microns long, to *A. pipistrelli* Macy, 1940, having numerous atrial spines 25 microns in length. Thus, if these spines were absent or overlooked, any specimen would necessarily be placed in *Prosthodendrium*. In the writer's opinion, the distinction between *Acanthatrium* and *Prosthodendrium* is not sufficient to warrant existence of both genera. On the other hand, *Acanthatrium* as a subgeneric concept appears to have sufficient merit to justify it.

The writer concurs with Cheng in suppressing *Mesothatrium*, both as a genus and subgenus, because mere position of vitellaria is not a reliable basis for distinguishing supraspecific groups. This point has been discussed by Etges (1953) with respect to genera of the family Microphallidae, where extensive intraspecific variation in the vitellaria was demonstrated in *Maritrema obstipum*.

Life history studies reported by Brown (1926), Abdel-Azim (1936), Seitner (1945), Koga (1954), as well as the present account, are all in sufficient agreement to indicate that Dubois (1955) is correct in transferring the several species of *Acanthatrium* to *Prosthodendrium* Dollfus, 1931. Accordingly, *Acanthatrium amphidymum* Cheng, 1957, and *A. oligacanthum* Cheng, 1957, are transferred to *Prosthodendrium* as *Prosthodendrium amphidymum* and *P. oligacanthum*, in the

subgenus *Acanthatrium* Skarbilovich, 1948.

SUMMARY

1. Descriptions of adult, sporocyst, cercaria, and metacercaria stages of *Prosthodendrium* (*Acanthatrium*) *anaplocami* n. sp. are given.

2. Evidence is presented for the experimental life history of *P. anaplocami*. The snail, *Anaplocamus dilatatus* (Conrad), and the mayfly, *Hexagenia bilineata* (Say), were found infected in the field; mayflies and laboratory mice were both infected experimentally.

3. Failure of the metacercariae of this species to encyst in mayfly nymphs is pointed out; encystment occurs only when the insects have undergone subimaginal ecdysis.

4. Since *Acanthatrium* Faust, 1919, has been suppressed, *Acanthatrium amphidymum* and *A. oligacanthum* are transferred to the genus *Prosthodendrium* Dollfus, 1931.

ACKNOWLEDGMENT

Expression of thanks is made here to Dr. Joseph P. E. Morrison, Division of Mollusks of the Smithsonian Institution, for his invaluable aid in identification of the snails used in this study.

REFERENCES CITED

ABDEL, AZIM, M. 1936 On the life history of *Lecithodendrium pyramidum* Looss, 1896, and its development from a xiphidiocercaria, *C. pyramidum* sp. nov. from *Melania tuberculata*. Ann. Trop. Med. Parasit. **30:** 351–356.

BROWN, F. J. 1926 Some freshwater larval trematodes, with contributions to their life histories. Parasitology **18:** 21–34.

CHENG, T. C. 1957 Studies on the genus *Acanthatrium* Faust, 1919 (Trematoda: Lecithodendriidae); with the description of two new species. J. Parasit. **43:** 60–65.

DUBOIS, G. 1955 Les trématodes de chiroptères de la collection Villy Aellen. Rev. Suisse Zool. **62:** 469–506.

ETGES, F. J. 1953 Studies on the life histories of *Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinseniella amnicolae* n. sp. (Trematoda: Microphallidae). J. Parasit. **39:** 643–662.

KOGA, Y. 1954 The life history of a new trematode encysted in the nymphs of stone flies. Kurume Med. J. **1:** 19–26.

SEITNER, P. G. 1945 Studies on five new species of xiphidiocercariae of the virgula type. J. Parasit. **31:** 272–281.

SKARBILOVICH, T. S. 1948 In Skrjabin, K.I., Trematodes of Animals and Man. Family Lecithodendriidae Odhner, 1911. Moskva Leningrad. **2:** 337–590.

SOGANDARES-BERNAL, F. 1956 Four trematodes from Korean bats with descriptions of three new species. J. Parasit. **42:** 200–206.

YAMAGUTI, S. 1958 Systema Helminthum. Vol. I: The Digenetic Trematodes of Vertebrates. Parts I and II. Interscience Publishers, N.Y.

EXPLANATION OF PLATE I

Stages in the life history of *Prosthodendrium* (*Acanthatrium*) *anaplocami* n. sp. Scaled in millimeters.

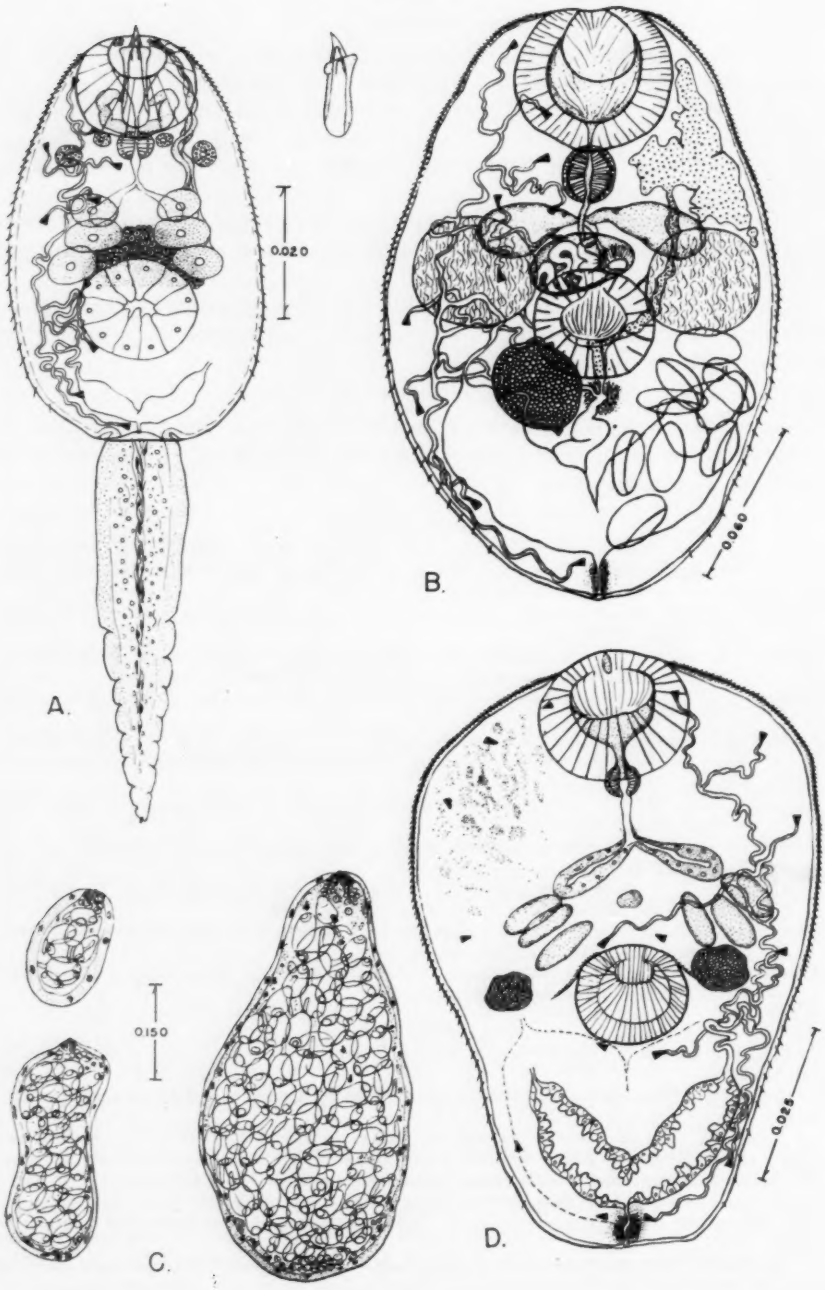
FIGURE A. The cercaria, drawn free-hand from living specimens, newly emerged from the snail.

FIGURE B. The adult worm, obtained from an experimental infection of a mouse; drawn from a stained permanent mount with the aid of a camera lucida; some details, such as flame cells, excretory tubules, and cuticular spines added from studies of living specimens.

FIGURE C. Three sporocysts dissected from gonad tissue of *Anaplocamus dilatatus* (Conrad); drawn with the aid of a camera lucida. Note the number of small developing cercariae in each.

FIGURE D. The unencysted metacercaria, drawn free-hand from living specimens dissected from the mayfly nymph, *Hexagenia bilineata* (Say). Note the reduced stylet and the extent of the expanded excretory bladder (indicated by dashed outline).

PLATE I



SOME DIGENETIC TREMATODES OF FISHES OF FRIDAY HARBOR, WASHINGTON*

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Since the publications of Guberlet and his students, there have been few investigations on the trematodes of marine fishes of the Puget Sound region. Lloyd and Guberlet (1932, 1936) named a new genus and species of Monorchiidae and described *Syncoelium filiferum* (Sars) from the Pacific salmon. McFarlane (1936) studied 15 species of trematodes, 7 of them new, from the more common shallow-water fishes in Departure Bay, B. C.; the hosts and parasites also occur in Puget Sound. Lloyd (1938) investigated trematodes from Seattle and Friday Harbor and reported 13 digeneans, including 3 new species. Acena (1947) described one new genus of hemiuroids and two new species of lepocreadiids; validity of the last two was questioned by Hanson (1950). Margolis and Adams (1956) and Margolis (1958) have recently reported two new hemiuroids from British Columbia waters.

Trematodes of fishes from the California coast are frequently found further north. Among them are 8 species of the genus *Podocotyle* described by Park (1937) from Dillon Beach. Annereaux (1943, 1947) collected 4 new species from fishes of southern California. Manter and Van Cleave (1951) reported 17 species from La Jolla and Montgomery (1957) recorded 22 additional species from the same area.

The following report includes 24 species of trematodes from 21 species of fishes of Friday Harbor.

MATERIALS AND METHODS

The trematodes were fixed and killed in AFA (alcohol-formalin-acetic acid) under a coverslip. They were stored in 70 percent alcohol and later stained with Delafield's hematoxylin or Semichon's carmine, cleared in xylol or clove oil, and mounted in permount.

Collections during the summers of 1957 and 1958 were made possible by grants from the National Science Foundation and the Wolcott Memorial Fund of the University of Nebraska. The work was conducted at the Friday Harbor Laboratories sponsored by the University of Washington. Acknowledgments are made to the personnel of the laboratories for use of facilities and aid in collections of fishes, and to Dr. H. W. Manter for suggestions and supervision during this study.

Fish identifications were made according to Clemens and Wilby (1949).

Types of all new species are deposited in the Helminthological Collection of the U. S. Museum. All measurements of trematodes are in millimeters unless indicated otherwise. Sucker ratios are transverse diameters of oral sucker: acetabulum, with the oral sucker as 1.

OBSERVATIONS

Family ZOOGONIDAE

1. *Steganoderma formosum* Stafford, 1904

Stafford (1904) and Manter (1926) both described *S. formosum* from *Hippoglossus hippoglossus* from the North Atlantic. Two specimens were collected by the

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author from *Parophrys vetulus* Girard, lemon sole (Pleuronectidae). The specimens differ from previous descriptions only in that the seminal vesicle is well-developed and divided into a small and large portion (fig. 1) and the vitellaria are more diffuse, not distinctly divided into follicles. The species is here reported for the first time on the Pacific coast of North America. The host record is new.

2. *Zoogonoides viviparus* (Olsson, 1868) Odhner, 1902

This species was found in the intestine of *Microstomus pacificus* (Lockington), Dover sole (Pleuronectidae). The single specimen is 1 by 0.43 in body size and has a sucker ratio of 1 : 1.5 instead of 1 : 2 as reported by Odhner (1905).

This species has been reported from Norway and England. One host in England, *Microstomus kitt* (Walbaum), is closely related to the one at Friday Harbor.

Family FELLODISTOMATIDAE

3. *Fellodistomum furcigerum* (Olsson, 1868) Yamaguti, 1953

One specimen was recovered from the intestine of *Pleuronichthys decurrens*, Jordan and Gilbert, curl-fin sole (Pleuronectidae). The species has been reported previously from Woods Hole and the Sea of Japan.

4. *Fellodistomum brevum* n. sp. (fig. 2)

Host: Microstomus pacificus (Lockington), Dover sole (Pleuronectidae).
Location: Intestine.
Frequency: One of 2 hosts examined.
Description (based on a single specimen) : Body plump, smooth, anterior end bluntly pointed, posterior end broadly rounded; length 1.221, width at acetabulum, 0.564. Oral sucker subterminal, 0.160 by 0.175; acetabulum with transverse aperture, 0.265 by 0.317, almost twice diameter of oral sucker, about one-third of body length from anterior end of body. Prepharynx lacking; pharynx 0.076 by 0.058; esophagus short, intestinal bifurcation dorsal to cirrus sac, close to oral sucker; ceca narrow, extending slightly posterior to testes. Genital pore to the left of intestinal bifurcation. Cirrus sac large, 0.245 by 0.150, obliquely-oriented in the area above acetabulum; containing bipartite seminal vesicle, well-developed prostate gland and short cirrus which becomes continuous with the folded walls of the genital atrium. Testes smooth, sub-spherical, 0.235 to 0.248 by 0.165 to 0.210, symmetrical, in posterior third of the body. Ovary multilobed, 0.240 by 0.200, on right, anterior to right testis, overlapping posterior edge of acetabulum. Seminal receptacle lacking. Vitellaria of small follicles in two lateral clusters in posterior half of body, reaching from posterior edge of acetabulum to that of testes, lateral to testes and mostly extracecal. Coils of uterus between, and posterior to, testes. Eggs yellow, 46 to 53 by 21 to 26 microns. Excretory bladder Y-shaped; stem branching at the anterior edge of testes, arms extending to region of pharynx.

Species of *Fellodistomum* with vitellaria in the acetabulo-testicular zone are: *F. furcigerum* (Olsson, 1868) Yamaguti, 1953; *F. magnum* (Manter, 1934) Yamaguti, 1953; and *F. profundum* (Manter, 1934) Yamaguti, 1953. In the other species of *Fellodistomum* the vitellaria extend anterior to the acetabulum. *F. brevum* resembles most *F. profundum* and *F. furcigerum* as drawn by Lebour (1908), but differs from them in the comparatively larger, rounded testes, and the shorter ceca which do not extend halfway between the testes and posterior end. The post-testicular region is short, only one-fifth of the body length, while it is one-third or more in *F. profundum* and *F. furcigerum*. This species differs further from *F. profundum* in the larger eggs, 46 to 53 by 21 to 26 microns instead of 30 to 34 by 16 microns; and in the absence of the uterus lateral to the ceca in the posterior half of the body. It differs from *F. furcigerum* in having a sucker ratio of 1 : 2 instead of 1 : 1.67, and folds of the genital atrium not mentioned or figured by Lebour.

Family LEPOCREADIIDAE

5. *Opechona alaskensis* Ward and Fillingham, 1934

This species was first reported from Alaska from a "toadfish." This host may have been the midshipman, *Porichthys notatus* Girard (Batrachoididae), which is the only species of the "toadfish" family in the northwest Pacific listed by Clemens and Wilby (1949).

One trematode fitting the description of *O. alaskensis* was collected from *Sebastodes ruberrimus* Cramer, red snapper (Scorpaenidae), near Henry Island. Small amounts of pigment in the anterior region of the prepharynx, finely granular gland cells in the parenchyma anterior to the vitellaria, and lobed testes were observed in this worm.

6. *Opechona occidentalis* Montgomery, 1957

McFarlane (1936) first reported *Opechona bacillaris* (= *Pharyngora bacillaris*) from *Sebastodes* sp. in British Columbia. Montgomery (1957) considered McFarlane's specimens to be *O. occidentalis*, perhaps a subspecies, because of the close agreement of characters such as the genital pore and inter-testicular vitellaria.

One specimen was collected from 3 *Sebastodes maliger* (Jordan and Gilbert), orange-spotted rock fish (Scorpaenidae). The trematode resembles those described by Montgomery from *Sebastodes atrovirens* (Jordan and Gilbert) in California even to the anterior extent of the vitellaria.

7. *Opechona parvasoma* n. sp. (fig. 3)

Host: Sebastodes melanops (Girard), black rock fish (Scorpaenidae).
Location: Intestine.
Frequency: Five specimens from 1 of 2 fish examined.
Description (measurements on 5 specimens, average in parentheses): Body small, flattened, anterior end tapering bluntly, posterior end rounded, widest posterior to the acetabulum with the last two-thirds of body approximately the same width; length 0.87 to 1.29 (1.15); width 0.23 to 0.43 (0.35). Cuticula heavily spined at the anterior end, becoming sparsely-spined posteriorly. Gland cells present in parenchyma anterior to acetabulum and lateral to digestive system. Pigment granules abundant in prepharynx region. Oral sucker trumpet-shaped, subterminal to terminal, 0.09 to 0.18 (0.14) by 0.08 to 0.12 (0.10). Acetabulum subcircular, about one-third body length from anterior end and slightly to right, 0.10 to 0.13 (0.11) in diameter. Sucker ratio 1 : 1.1. Prepharynx 0.13 long in one specimen but short in the other 4; pharynx 0.09 to 0.10 (0.09) by 0.04 to 0.06 (0.05). True esophagus short, pseudoesophagus 2 to 4 times longer than true esophagus, the length 0.08 to 0.14 (0.10). In the extended specimen, the prepharynx is one-sixth longer than the total esophagus, the total esophagus and pharynx being almost equal. Intestinal bifurcation immediately anterior to acetabulum, ceca reaching to posterior end of body. Genital pore to left of acetabulum. Testes smooth, wider than long, tandem, intercecal, in posterior third of body; posterior testis 0.05 to 0.22 from the posterior end of body. Cirrus sac bent sharply to left, contains cirrus, pars prostatica, numerous prostate cells, and small internal seminal vesicle. External seminal vesicle small, globular. Ovary somewhat irregular in shape, with 3 lobes, separated from anterior testis by yolk reservoir and seminal receptacle. Seminal receptacle ovoid, overlapping yolk reservoir ventrally; Laurer's canal long, extending posterior to ovary before turning dorsally; uterus preovarial, with muscular metraterm. Eggs operculate, thin, 64 to 70 by 33 to 39 microns. Vitellaria around the ceca, partially confluent posterior to testes, extending from posterior edge of cirrus sac to posterior end of body. Excretory vesicle a short tube, barely reaching posterior testis; excretory pore with sphincter.

O. parvasoma resembles *O. bacillaris* (Molin, 1859) Looss, 1907, and *O. occidentalis* Montgomery, 1957, in the extent of the vitellaria, trumpet-shaped oral sucker, and tri-lobed ovary. It differs from both in the short, broad body, the inconspicuous external seminal vesicle and the short excretory bladder which reaches only to the testes. In addition, *O. parvasoma* has almost contiguous rather than separated testes and eggs 10 microns shorter than those in *O. bacillaris*.

O. scombri Yamaguti, 1938, is described as having no pseudoesophagus. Since the generic diagnosis includes an esophagus partly lined with epithelium, this species should be transferred to *Lepocreadium,* becoming *L. scombri* (Yamaguti, 1938) n. comb. *O. girellae* Yamaguti, 1940, is described as having an esophagus 30 to 60 microns long, provided with circular and longitudinal muscle fibers and surrounded by accompanying cells. The esophagus is short and an epithelium is not pictured in it. In addition, numerous large prostate cells surround the external seminal vesicle and lie free in the parenchyma. For this reason, the species is transferred to *Neolepidapedon* Manter, 1954, becoming *N. girellae* (Yamaguti, 1940) n. comb.

Yamaguti (1953, 1958) lists *Lepidapedon levinseni* (Linton, 1907) Manter, 1947, as a species of *Opechona,* but notes Manter's (1947) placement of the species in *Lepidapedon.* This species has the bipartite cirrus sac of *Lepidapedon* and no pseudoesophagus.

8. *Lepidapedon microcotyleum* (Odhner, mss) Dollfus, 1953 (fig. 4)

Host: Theragra chalcogramma (Pallas), whiting (Gadidae).
Location: Intestine.
Frequency: One of 4 hosts examined.
Description (measurements on a single specimen) : Body elongate, spined, length 1.83, width at level of acetabulum 0.43. Oral sucker nearly round, 0.09; acetabulum slightly larger, 0.10 in diameter, about one-third body length from anterior end, 0.19 from intestinal bifurcation. Prepharynx and esophagus equal in length, 0.04; pharynx 0.08 by 0.06. Glandular cells in the parenchyma anterior to the intestinal bifurcation; intestinal ceca narrow, ending blindly at the posterior end of body. Testes smooth, ovoid, tandem, separated by vitellaria. Post-testicular space 0.21 or about one-ninth of body length. Cirrus sac large and ovoid, dorsal to acetabulum and slightly anterior to it. Genital pore on the left, closely anterior to acetabulum. Internal seminal vesicle narrow, external seminal vesicle sinuous, surrounded by a membranous sac with gland cells few and mainly at beginning of external seminal vesicle. Ovary smooth, wider than long, in middle of the hind body, separated from anterior testis by the saccate yolk reservoir and vitellaria. Seminal receptacle directly posterior to ovary, slightly to right; Laurer's canal turns posterior to ovary before opening dorsally. Vitellaria from just posterior to acetabulum to posterior end of body, filling the lateral, inter- and post-testicular areas, overlapping ceca dorsally and ventrally. Uterus preovarian, intercecal; metraterm muscular. Eggs thin-shelled, somewhat collapsed, 64 by 34 microns. Excretory vesicle, a narrow tube, reaching the anterior edge of anterior testis.

Dollfus (1953) described and figured "*Lepidapedon microcotyleum* (Odhner, mss)" from 2 specimens from the Atlantic cod, *Gadus callarias,* at Thorshavn (Feroe) and from 11 specimens from the cod collected by Odhner at Kristineberg (Sweden). Odhner's slides were labeled either *Lepodora* n. sp. or *Lepodora microcotylea* Odhn., but no description or figure was ever published under the latter name until Dollfus' work. The "manuscript" name of Odhner consists of a labeled slide.

Dollfus also considers the following to be *L. microcotyleum: "Distomum* sp." described by Linton (1901) from *Enchelyopus cimbrius* (L.) at Woods Hole, Mass.; "*Lepodora* neue Art" of Odhner (1905) from the cod off the west coast of Sweden; "*Lepidapedon elongatum* Lebour" of Dogiel (1936) from the cod in Barents Sea; "*Lepidapedon elongatum* (Lebour)" of Linton (1940) in the cod, *Enchelyopus cimbrius* (L.), *Pollachius virens* (L.), and *Urophycis chuss* (Walbaum) from Woods Hole.

The species differs from *L. elongatum* (Lebour, 1908) Nicoll, 1915, in the greater separation of the gonads by invasion of the vitellaria between them and in the post-testicular area. *L. microcotyleum* with retracted anterior end as drawn by Dogiel (1936) is almost identical to the author's specimen and one labeled "*Lepi-*

dapedon elongatum from *Urophycis chuss* (?)" in Manter's 1934 Tortugas, Florida, collection. The distribution of the trematode appears to be from the deep waters of Tortugas, Florida, to the Arctic and to the Pacific Northwest in gadoid fishes.

The description of *L. clavatum* Linton, 1940, does not mention the nature of the seminal vesicles and they are indistinct in the figure given. The species should be regarded as *incertae sedis.*

Family OPECOELIDAE

9. *Podocotyle sinusacca* n. sp. (figs. 5, 6)

Host: Leptocottus armatus Girard, cabezon (Cottidae).
Location: Intestine.
Frequency: Six specimens in one of two mature hosts examined.
Description (measurements on 5 of 6 specimens, average in parentheses): Body smooth, very elongate, tapering to a blunt point at posterior end; length 2.40 to 2.92 (2.67), width at the level of ovary, 0.16 to 0.32 (0.27). Oral sucker terminal to subterminal, 0.15 to 0.16 (0.16) in diameter. Sucker ratio 1 : 1.4. Acetabulum 0.18 to 0.26 by 0.22 to 0.28 (0.22 by 0.26), about one-sixth to one-seventh from the anterior end of body, 0.36 to 0.72 (0.50); borne on a stalk which may project as far as 0.57 from the body. Prepharynx short, pharynx almost round, 0.07 to 0.14 (0.10) in diameter; esophagus 0.13 to 0.17 (0.15) long; intestinal ceca almost reaching posterior end of body. Genital pore to left of intestinal bifurcation; cirrus sac very long, sinuate, extending halfway between the acetabulum and ovary; cirrus protrusible. Seminal vesicle constricted into a small anterior part and a large posterior part, the narrow connection between the two folded once, and overlapping the posterior part (fig. 3). Testes tandem, ovoid and slightly lobed; anterior testis 0.20 to 0.25 by 0.15 to 0.20 (0.17 by 0.23); posterior testis 0.11 to 0.20 by 0.20 to 0.27 (0.17 by 0.25); intertesticular space 0.17 to 0.30 (0.21). Ovary 4-lobed, with 3 lobes posteriorly directed, antero-lateral to anterior testis, usually on the right, 0.12 to 0.15 by 0.12 to 0.15 (0.14 to 0.14).

Oviduct arises from the anterior lobe of the ovary, joined near its middle by the pear-shaped seminal receptacle. Laurer's canal near anterior end of seminal receptacle, turns posteriorly and then dorsally. Vitellaria of round follicles beginning 0.60 to 0.86 (0.75) from anterior end of body, mostly lateral, interrupted at each testis, filling post-testicular space. Eggs oval with one blunt end, 69 to 79 microns by 26 to 32 microns. Excretory bladder extending to level of ovary, excretory pore terminal.

Over 30 species of *Podocotyle* have been named. *P. sinusacca* keys to *P. endophrysi* Park, 1937, in Park's key. It resembles *P. endophrysi* in the protrusion of the anterior part of the cirrus, but differs in the arrangement of the seminal vesicle within the cirrus sac and in the separation of the testes. In body form, *P. sinusacca* is similar to *P. pacifica* Park, 1937, and *P. olssoni* Odhner, 1905, but differs in the sinuous cirrus sac and the arrangement of the seminal vesicle which is coiled in *P. olssoni* and narrowly looped once in *P. pacifica.*

10. *Podocotyle pedunculata* Park, 1937

Host: Leptocottus armatus Girard, cabezon (Cottidae).
Location: Intestine.
Frequency: One of 2 hosts examined.

Nine specimens were found in the same host with *P. sinusacca* which were readily distinguished by the straight, short cirrus sac. The oral sucker and pharynx of *P. pedunculata* were larger than the range given by Park and the peduncle was more pronounced. *P. pedunculata* and *P. kofoidi* Park, 1937, are very similar, but the latter differs in the length of esophagus, larger size and more lobed testes. Although some of my specimens show a slight lobation of the testes, this condition is not as distinct as in *P. kofoidi.*

11. *Podocotyle pacifica* Park, 1937

Host: Gasterosteus aculeatus (L.), 3-spined stickleback (Gasterosteidae).

Location: Intestine.
Frequency: One to 38 in 15 hosts examined.

The single difference between *P. pacifica* and *P. olssoni* Odhner, 1905, appears to be the arrangement of the seminal vesicle which is constantly sinuous in the former and coiled in the latter.

12. *Podocotyle abitionis* McFarlane, 1936

One specimen was collected from *Sebastodes* sp., the host reported by McFarlane. As described by him, the cirrus sac has a portion coiled posteriorly; however, the seminal vesicle within is straight.

Superfamily HEMIUROIDEA

Special effort was made to determine the distribution of hemiuroids in fishes of Friday Harbor. None was new; the most abundant in number and widest in host distribution was *Tubulovesicula lindberghi* (Layman, 1930) Yamaguti, 1934 (201 specimens found in 13 hosts); followed by *Lecithaster salmonis* Yamaguti, 1934 (81 in 9 hosts); *Derogenes varicus* (Mueller, 1784) (36 in 8 hosts); and *Lecithochirium exodicum* McFarlane, 1936 (36 in 3 hosts).

13. *Lecithaster salmonis* Yamaguti, 1934

Many specimens were collected and most of them were examined alive. One immature specimen was found in a cyclopoid copepod from a plankton haul. It showed the typical 4-lobed ovary and rosette-shaped vitellaria. Both immature and mature specimens were particularly abundant in young sticklebacks and *Sebastodes* sp.

Specimens were found near and below the minimum sizes given by Yamaguti (1938) as 0.95 and by Lloyd (1938) as 0.60. Egg measurements on preserved material were smaller than those by Yamaguti made on live specimens. Nine specimens from 0.43 to 0.7 long had an almost constant sucker ratio of 1 : 1.5. In 6 specimens over 0.7 in length, the ratio varied from 1 : 1.7 to 1 : 2.0.

Characteristics of this species are: the wide body, elongate seminal vesicle reaching to the posterior edge of acetabulum or beyond, the long pars prostatica with abundant gland cells, and eggs 21 to 24 by 13 to 16 microns. In my specimens, small papillae on the oral and acetabular surfaces were observed on live specimens and some preserved ones; they were not mentioned by Yamaguti, but were noted in another species, *Lecithaster confusus* Odhner, 1905, by Hunninen and Cable (1943). The excretory vesicle widens just before opening at the terminal excretory pore. Anteriorly, it branches just posterior to the acetabulum, the lateral branches continuing to the sides of the pharynx. An anterior commissure was not observed. Lloyd (1938) mentioned that the lobes of the vitellaria were thicker than indicated by Yamaguti and this tendency was true for most of my specimens.

The number of lobes of the ovary and vitellaria was often difficult to determine because of eggs. In at least 2 specimens, there were 6 lobes of vitellaria instead of 7 and in one, only 3 lobes of the ovary instead of 4. Two specimens showed shorter ceca which did, however, extend to the level of the vitellaria. In view of the constant characters such as the shape and extent of the seminal vesicle and egg sizes in all specimens, these differences are regarded as individual variations.

There follows a summary of fish trematodes collected from 97 hosts belonging to 21 species. New host records are indicated by an asterisk.

BUCEPHALIDAE

Prosorhynchus apertus McFarlane, 1936. Host: *Ophiodon elongatus* (Girard).

Rhiphidocotyle elongatum McFarlane, 1936. Host: *Ophiodon elongatus* (Girard).

APOROCOTYLIDAE

Aporocotyle simplex (Odhner, 1900). Host: *Sebastodes maliger* (Jordan and Gilbert).

ACANTHOCOLPIDAE

Stephanostomum tristephanum McFarlane, 1936. Host: *Ophiodon elongatus* (Girard).

ZOOGONIDAE

Zoogonoides viviparus (Olsson, 1868) Odhner, 1902. * Host: *Microstomus pacificus* (Lockington).

Steganoderma formosum Stafford, 1904. * Host: *Parophrys vetulus* Girard.

FELLODISTOMATIDAE

Fellodistomum furcigerum (Olsson, 1868) Yamaguti, 1953. * Host: *Pleuronichthys decurrens* (Jordan and Gilbert).

Fellodistomum brevum n. sp. Host: *Microstomus pacificus* (Lockington).

LEPOCREADIIDAE

Opechona alaskensis Ward and Fillingham, 1934. * Host: *Sebastodes ruberrimus* Cramer.

O. occidentalis Montgomery, 1957. * Host: *Sebastodes maliger* (Jordan and Gilbert).

O. parvasoma n. sp. Host: *Sebastodes melanops* (Girard).

Lepidapedon microcotyleum (Odhner, mss) Dollfus, 1953. * Host: *Theragra chalcogramma* (Pallas).

OPECOELIDAE

Opecoelina theragrae Lloyd, 1938. * Host: *Sebastodes maliger* (Jordan and Gilbert).

Podocotyle abitionis McFarlane, 1938. Host: *Sebastodes maliger* (Jordan and Gilbert).

P. pacifica Park, 1937. * Host: *Gasterosteus aculeatus* (L.)

P. pedunculata Park, 1937. * Host: *Leptocottus armatus* Girard.

P. sinusacca n. sp. Host: *Leptocottus armatus* Girard.

HEMIUROIDEA

Lecithaster salmonis Yamaguti, 1934. Hosts: * *Sebastodes maliger* (Jordan and Gilbert); * *S. melanops* (Girard); * *Leptocottus armatus* Girard; * *Clinocottus embryum* Jordan and Starks; * *Oncorhynchus kisutch* (Walbaum); * *Gasterosteus aculeatus* (L.); * *Cymatogaster aggregatus* Gibbons; * *Porichthys notatus* Girard.

Derogenes varicus (Mueller, 1784). Hosts: *Sebastodes maliger* (Jordan and Gilbert); * *S. caurinus* (Richardson); *Ophiodon elongatus* (Girard); * *Clinocottus embryum* Jordan and Starks; * *Platichthys stellatus* (Pallas); * *Gasterosteus aculeatus* (L.); * *Porichthys notatus* Girard; * *Isopsetta isolepis.*

Genolinea manteri Lloyd, 1938. * Host: *Dasycottus setiger* Bean.

Tubulovesicula lindberghi (Layman, 1930) Yamaguti, 1934. Hosts: * *Sebastodes caurinus* (Richardson); * *S. melanops* (Girard); * *S. nigrocinctus* (Cramer); * *Ophiodon elongatus* (Girard); * *Leptocottus armatus* Girard; * *Hemilepidotus hemilepidotus* (Tilesius); * *Synchirus gilli* Bean; * *Isopsetta isolepis* (Lockington); *Parophrys vetulus* Girard; * *Theragra chalcogramma* (Pallas); * *Oncorhynchus kisutch* (Walbaum); * *Gasterosteus aculeatus* (L.).

Lecithochirium exodicum McFarlane, 1936. Hosts: *Sebastodes maliger* (Jordan and Gilbert); *Ophiodon elongatus* (Girard); * *Platichthys stellatus* (Pallas).

Hemiurus levinseni Odhner, 1905. Host: * *Theragra chalcogramma* (Pallas).

Parahemiurus merus (Linton, 1910) Woolcock, 1930. Host: * *Gasterosteus aculeatus* (L.).

GEOGRAPHICAL DISTRIBUTION OF FISH TREMATODES OF FRIDAY HARBOR

Of the 42 species of Digenea reported from the Friday Harbor area, 23 have been found in fishes elsewhere in one or more major localities. Four are found farther south in California, 1 farther north in Alaska. Thirteen are found in the North Atlantic; 4 have been reported from Tortugas, Florida, or the South Atlantic, and 7 are from Japan. Greatest affinities appear to be with trematodes of the North Atlantic and Japan.

SUMMARY

Twenty-four species of digenetic trematodes were collected from 21 species of Friday Harbor fishes. Three are regarded as new: *Fellodistomum brevum* (Fellodistomatidae) from *Microstomus pacificus* (Lockington), Dover sole; *Opechona*

parvasoma (Lepocreadiidae) from *Sebastodes melanops* (Girard), black rock fish; *Podocotyle sinusacca* (Opecoelidae) from *Leptocottus armatus* Girard, cabezon.

Opechona scombri Yamaguti, 1938, is transferred to *Lepidapedon*, and *Opechona girellae* Yamaguti, 1940, is transferred to *Neolepidapedon*.

Thirty-eight new host records are indicated for 16 species of Digenea; the majority of new hosts were for hemiuroids. The geographical distribution of trematodes found in the Friday Harbor area shows that strongest links are with the North Atlantic and Japan.

Literature Cited

Acena, S. P. 1947 New trematodes from Puget Sound fishes. Trans. Am. Micr. Soc. **66:** 127–139.

Annereaux, R. F. 1943 *Opecoelina pharynmagna* n. sp. (Trematoda) from the China rockfish. J. Parasit. **29:** 155–156.

———1947 Three new trematodes from marine fishes of California. Trans. Am. Micr. Soc. **66:** 249–255.

Clemens, W. A. and Wilby, G. V. 1949 Fishes of the Pacific coast of Canada. Fish. Res. Bd. Can., Bulletin 68.

Dogiel, V. A. 1936 (Parasites of cod of Lake Mogil'noe.) Uchen. Zapiski Leningrad. Gosudarstv. Univ. Bubnov (7), s. Biol. (3), 124–133. (In Russian.)

Dollfus, R. P. 1953 Aperçu général sur l'histoire naturelle des parasites animaux de la morue atlanto-arctique *Gadus callarias* L. (= *morphua* L.). Encycl. Biol. **43.**

Hanson, M. L. 1950 Some digenetic trematodes of marine fishes of Bermuda. Proc. Helm. Soc. Wash. **17:** 74–88.

Hunninen, A. V. and Cable, R. M. 1943 The life history of *Lecithaster confusus* Odhner (Trematoda: Hemiuridae). J. Parasit. **29:** 71–79.

Lebour, M. V. 1908 Fish trematodes of the Northumberland coast. Rep. Northumb. Sea Fish. Com., 1907, pp. 23–67.

Linton, E. 1901 Parasites of fishes in the Woods Hole region. Bull. U. S. Fish. Com. 1900, pp. 405–422.

———1940 Trematodes from fishes mainly from the Woods Hole region, Massachusetts. Proc. U. S. Nat. Mus. (3078) **88:** 1–172.

Lloyd, L. C. 1938 Some digenetic trematodes from Puget Sound fish. J. Parasit. **24:** 103–133.

——— and Guberlet, J. E. 1932 A new genus and species of Monorchidae. J. Parasit. **18:** 232–239.

——— and Guberlet, J. E. 1936 *Syncoelium filiferum* (Sars) from the Pacific salmon. Trans. Am. Micr. Soc. **55:** 44–48.

McFarlane, S. H. 1936 A study of the endoparasitic trematodes from marine fishes of Departure Bay, B. C. J. Biol. Bd. Can. **2:** 335–347.

Manter, H. W. 1926 Some North American fish trematodes. Ill. Biol. Monog. **10:** 1–138.

———1947 The digenetic trematodes of marine fishes of Tortugas, Florida. Am. Midl. Nat. **38:** 257–416.

——— and Van Cleave, H. J. 1951 Some digenetic trematodes, including eight new species, from marine fishes of La Jolla, Calif. Proc. U. S. Nat. Mus. (3279) **101:** 315–339.

Margolis, L. 1958 A new species of *Lecithophyllum* from North Pacific fishes with a consideration of the taxonomy of the genera *Lecithophyllum, Aponurus,* and *Brachadena* (Trematoda: Hemiuridae). Can. J. Zool. **36:** 893–905.

——— and Adams, J. R. 1956 Description of *Genolinea oncorhynchi* n. sp. (Trematoda: Hemiuridae) from *Oncorhynchus gorbuscha.* Can. J. Zool. **34:** 573–577.

Montgomery, W. R. 1957 Studies on digenetic trematodes from marine fishes of La Jolla, California. Trans. Am. Micr. Soc. **76:** 13–36.

Odhner, T. 1905 Die Trematoden des Arktischen Gebietes. Fauna Arct., Jena **4:** 291–372.

Park, J. T. 1937 A revision of the genus *Podocotyle* (Allocreadinae) with descriptions of eight new species from tidepool fishes from Dillon's Beach, Calif. J. Parasit. **23:** 405–422.

Stafford, J. 1904 Trematodes from Canadian fishes. Zool. Anz. **28:** 681–694.

Yamaguti, S. 1953 Systema Helminthum. Part I. Digenetic Trematodes of Fishes. Maruzen Co., Tokyo.

———1958 Systema Helminthum. Vol. I. The Digenetic Trematodes of Vertebrates. Interscience, New York.

EXPLANATION OF PLATE I.

FIGURE 1. Terminal genitalia of *Steganoderma formosum*. (gp, genital pore; mt, metraterm; sv, seminal vesicle).
FIGURE 2. *Fellodistomum brevum* n. sp., ventral view.
FIGURE 3. *Opechona parvasoma* n. sp., ventral view.
FIGURE 4. *Lepidapedon microcotyleum*, ventral view.
FIGURE 5. *Podocotyle sinussacca* n. sp., ventral view.
FIGURE 6. Part of cirrus sac, *P. sinussacca*. (cs, cirrus sac; sv, seminal vesicle).

RESEARCH NOTE

A TECHNIQUE FOR MAINTENANCE OF THE SNAIL *LITTORINA PINTADO* IN THE LABORATORY.

The littorine snail, *Littorina pintado* Wood, an intermediate host of *Austrobilharzia variglandis* Penner, has a natural habitat of rocky surfaces in the intertidal zone along Hawaiian sea shores. Until recently attempts to maintain these snails in an ordinary aquarium for a long period usually ended in failure. Recently a new maintenance method has been developed and now these snails may be used for laboratory expriments with *A. variglandis*.

Medium to large snails were collected from rocky cliffs or sea benches about Oahu and Rabbit Islands. As far as possible, the schistosome-infected snails were separated from the "clean" ones in the laboratory by determining their capacity to release cercariae upon contact with sea water. These specimens constituted the experimental material for tests with this new method of maintenance.

The technique involved the use of wide-mouth gallon jars to house the amphibious snails. The jars may be inexpensively obtained after their use as food containers. Usually 20 to 40 snails were placed in each jar. Sea water was added to a depth of approximately 5 cm and the jar was covered with an inverted 8-inch finger bowl. The two pieces were then carefully turned over and more sea water was immediately added to the finger bowl to equalize the level in both containers. Thus, the snails were enclosed in a humid chamber over a reservoir of water. To keep the "aquaria" in a sanitary state, the sea water was changed twice a week.

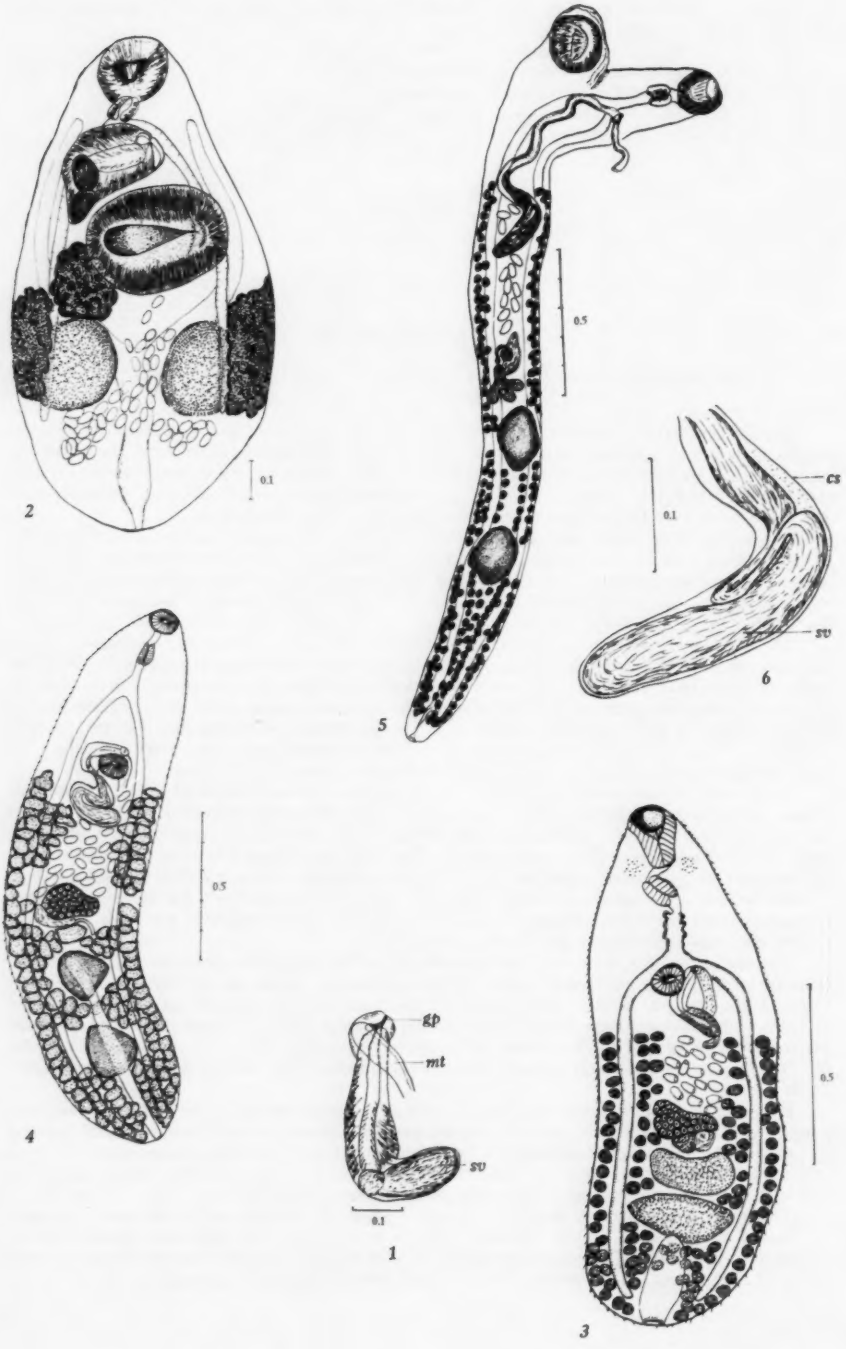
An altrnate method which also worked effectively was the use of inverted 1-liter Erlenmeyer flasks. Each flask was fitted with a single-hole No. 9 rubber stopper into which was inserted one arm (12 cm) of a U-shaped glass tube (9mm O.D.). Ten snails were then placed in the flask after 100 ml of sea water were added. The flask was closed with the prepared stopper and inverted so that water could not enter the glass tubing. Such inverted flasks were held in place by iron ring stands or wooden racks. It appeared that provision for gaseous exchange through the tube made it necessary to change the sea water less frequently, generally only once a week, unless putrefaction of dead snails occurred.

The success of these devices relied much on the willingness of the snails to feed. The snail food employed in these expriments was a slight modification of that reported by Lee and Lewert (1956, J. Inf. Dis. **99**: 15–20). Fifty percent more water was mixed with the ingredients listed by them. The food was kept frozen in squeeze-type plastic bottles. A thin coating of this food preparation was applied to the bottom of a gallon jar or liter flask and permitted to air dry for 24 hours before the snails and sea water were added. The nutrient thus furnished lasted about 2 weeks.

By means of these methods, the "clean" snails were maintained for as long as 9 months, with a mortality rate of less than 20 percent. Snails naturally infected with schistosomes and releasing cercariae from time to time did not live for more than 1 to 2 months. Experiments are now in progress to study the survival of snails infected with schistosome miracidia in the laboratory and kept in the inverted containers at room temperature (25–30°C).

This work was supported in part by research grant No. E-2424, of the National Institutes of Health, U. S. Public Health Service. This is contribution No. 121 from Hawaii Marine Laboratory, University of Hawaii.—GEORGE W. T. C. CHU AND EDWARD PARSONS RYAN, *Departments of Bacteriology and Zoology, University of Hawaii, Honolulu, Hawaii.*

PLATE I



STUDIES ON SOME CESTODE PARASITES. V. TWO NEW SPECIES OF CESTODES BELONGING TO THE FAMILY *HYMENOLEPIDIDAE* FUHRMANN, 1907

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During the years 1953 to 1956, the author examined a total of twenty-six vampire bats, *Pteropus medius* Temminck, from Bareilly, Uttar Pradesh. One was found infected with a large number of minute cestodes of the genus *Hymenolepis*.

Hymenolepis minimedius n. sp.

Diagnosis: Maximum length of the strobila 16–25 mm, maximum width 0.96 mm. Scolex small (pl. I, fig. A), 0.16 to 0.18 mm by 0.24 to 0.28 mm, slightly set off from the neck. Suckers rounded or oval, 0.085 to 0.10 mm by 0.09 mm. Rostellum prominent and unarmed, 0.125 to 0.140 mm by 0.06 to 0.08 mm. Rostellar pouch absent. Neck short, 0.06 to 0.075 mm in length, with a maximum width of 0.028 mm. Margins of the strobila serrate. All segments broader than long. Immature segments 0.05 mm by 0.34 to 0.37 mm, mature ones 0.22 mm by 0.68 mm (pl. I, fig. B), and the gravid ones 0.32 mm by 0.9 mm. Genital pores unilateral, dextral and located a little behind the middle of the margin of the segments. Genital atrium absent. Cirrus sac elongated 0.16 to 0.20 by 0.02 to 0.035 mm, extending dorsal to and much beyond ventral poral longitudinal excretory vessel and approximately one-fifth across the segment. Cirrus unarmed. Vas deferens enlarged into external vesicula seminalis, 0.12 mm by 0.06 mm, extending to the middle of the segment. Testes 3 (diameter 0.086 to 0.092 mm), arranged almost in a straight line, 1 poral and 2 aporal in position. Ovary slightly bilobed, elongate and transversely situated in the middle of the anterior part of the segment. It is 0.18 mm across its widest part. Vitelline gland transversely elongated just posterior to the ovary. Receptaculum seminis absent. Vagina opening posterior to the male genital opening. Uterus sac-like and lobed, filling the entire segment. Eggs 0.05 mm by 0.056 mm, (pl. I, fig. C). Oncospheres 0.04 mm by 0.037 mm. Embryonic hooks 0.024 mm in length.

Type host: Pteropus medius Temminck.

Habitat: Intestine.

Type locality: Bareilly, Uttar Pradesh, India.

There are 13 species of *Hymenolepis* reported from bats of which only *H. grisea* (van Beneden, 1873) Joyeux and Baer, 1936, from Europe is unarmed. So far as the author has been able to ascertain, no species of *Hymenolepis* from bats has been reported from India. The present form differs from *H. grisea* in the size of the strobila, rostellum, cirrus sac, and the arrangement of the testes. Among the other species of *Hymenolepis* without rostellar hooks, *H. citelli* McLeod, 1933, and *H. rhodesiensis* Baer, 1933, are the only forms resembling the present form. The present form can be easily differentiated from these species by the size of the strobila, scolex, suckers and the arrangement of the testes. It also differs from *H. rhodesiensis* by the absence of a spiny cirrus. (See Joyeux and Baer, 1936; McLeod, 1933; Baer, 1933.)

Diorchis balacca n. sp.

About two dozen cestodes of this species were collected from the intestine of the common coot, *Fulica atra* Linn., captured in the environs of Lucknow.

Diagnosis: Maximum length of the strobila 72 to 96 mm, maximum width 0.71 mm attained in the region of the mature segments. Scolex small, slightly elongated and muscular, 0.46 to 0.53

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mm by 0.24 to 0.30 mm, passing insensibly into the neck, (pl. II, fig. A). Suckers muscular, rounded or oval, 0.18 to 0.22 mm in diameter. Rostellum muscular 0.18 to 0.20 mm by 0.09 to 0.11 mm. Rostellar sac large, 0.46 mm by 0.20 mm, extending much beyond the base of the suckers. Rostellum provided with a single row of 10 hooks, 0.062 to 0.065 mm long, the handle being much larger than the blade (pl. II, fig. C). Neck 6.3 mm by 1.6 mm. Dorsal and ventral longitudinal excretory vessels 0.0085 mm and 0.015 mm in diameter, respectively. All segments broader than long. Immature segments numerous, 0.05 mm by 0.34 mm, mature ones (pl. II, fig. B), 0.18 to 0.20 mm by 0.64 to 0.67 mm. The boundaries of the gravid segments are not clearly marked owing to the extension of the uterus. Genital pores unilateral situated near or at the middle of the margin of the segments. Cirrus sac (0.35 to 0.45 mm by 0.04 to 0.05 mm) slender and elongated extending beyond the poral longitudinal excretory vessels and much beyond the middle of the segment. External vesicula seminalis rounded or oval and variable in size. Testes 2 (diameter 0.10 to 0.12 mm); 1 near the poral and the other near the aporal margins of the segment between the longitudinal excretory vessels. Ovary slightly elongated and compact, situated near the middle of the segment, measuring 0.21 to 0.25 mm by 0.09 to 0.11 mm. Vitelline gland compact, 0.08 mm in diameter, posterior to the ovary, generally between the testes. Vagina ventral to cirrus sac, slightly dilated and not clearly demarcated from receptaculum seminis which develops early along with the male genital organs. Uterus saccular, slightly lobed and when fully developed extending much beyond longitudinal excretory vessels. Eggs (pl. II, fig. D) elongated and spindle-shaped, 0.065 mm by 0.018 mm. Onchospheres 0.028 mm by 0.015 mm. Embryonic hooks hardly visible.

Type host: *Fulica atra* Linn.

Habitat: Intestine.

Type locality: Lucknow, Uttar Pradesh, India.

After a detailed survey of all species of *Diorchis* Clerc, 1903, only *D. spinata* Mayhew, 1929, *D. visayana* Tubangui and Masilungan, 1937, and *D. spiralis* Szpotańska, 1931, appear to resemble the present form (See Mayhew, 1929; Tubangui and Masilungan, 1937; Szpotańska, 1931). *D. balacea* n. sp. differs from *D. spinata* in possessing larger rostellar hooks and in the larger size and greater extension of the cirrus sac into the segment; from *D. visayana* in the number of rostellar hooks (10 in *D. balacea* against 8 in *D. visayana*); from *D. spiralis* by the larger cirrus sac and the absence of a spiral sphincter in the vagina, *D. spiralis* being characterized by a small cirrus sac and the vaginal sphincter.

SUMMARY

Two new hymenolepidid cestodes have been described and figured: *Hymenolepis minimedius* n. sp. from *Pteropus medius* (Chiroptera), the first record of a hymenolepidid cestode from bats in India; and *Diorchis balacea* n. sp. from the common coot *Fulica atra* (aves).

ACKNOWLEDGMENTS

The author wishes to express his grateful appreciation to Professor M. B. Lal for his keen interest in guiding the present work which forms a part of the Ph.D. thesis, and to the authorities of the Lucknow University for allowing it to be published.

LITERATURE CITED

BAER, J. G. 1933 Contribution à l'étude de la faune helminthologique africaine. Rev. Suisse Zool. **40:** 31–84.

JOYEUX, C. E. AND BAER, J. G. 1936 Cestodes. Faune de France 30. Paris.

MAYHEW, R. L. 1929 The genus *Diorchis*, with description of four new species from North America. J. Parasit. **15:** 251–258.

MCLEOD, J. A. 1933 A parasitological survey of the genus Citellus in Manitoba. Can. J. Res. **9:** 108–127.

SZPOTAŃSKA, I. 1931 Quelques especès nouvelles ou peu connues des Hymenolepididae Fuhrmann (Cestodes). Ann. Mus. Zool. Polon. **9:** 247–266.

TUBANGUI, M. A. AND MASILUNGAN, V. A. 1937 Tapeworm parasites of Philippine birds. Phil. J. Sci. **62**: 409–438.

EXPLANATION OF PLATES

(All drawings made with the aid of a camera lucida)

PLATE I. FIGURE A. Scolex of *H. minimedius.*
FIGURE B. Mature segment.
FIGURE C. Egg.

PLATE II. FIGURE A. Scolex of *D. balacea.*
FIGURE B. Mature segment.
FIGURE C. Rostellar hook (enlarged).
FIGURE D. Egg.

ABBREVIATIONS USED: c.s., cirrus sac; d.ex.v., dorsal longitudinal excretory vessel; e.h., embryonic hook; ext.v.s., external vesicular seminalis; g.p., genital pore; onch., onchosphere; ov., ovary; r.h., rostellar hook; rost., rostellum; rost.s., rostellar sac; r.s., receptaculum seminis; suck., sucker; t., testis; v., vagina; v.ex.v., ventral longitudinal excretory vessel; v.gl., vitelline gland.

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PLATE I

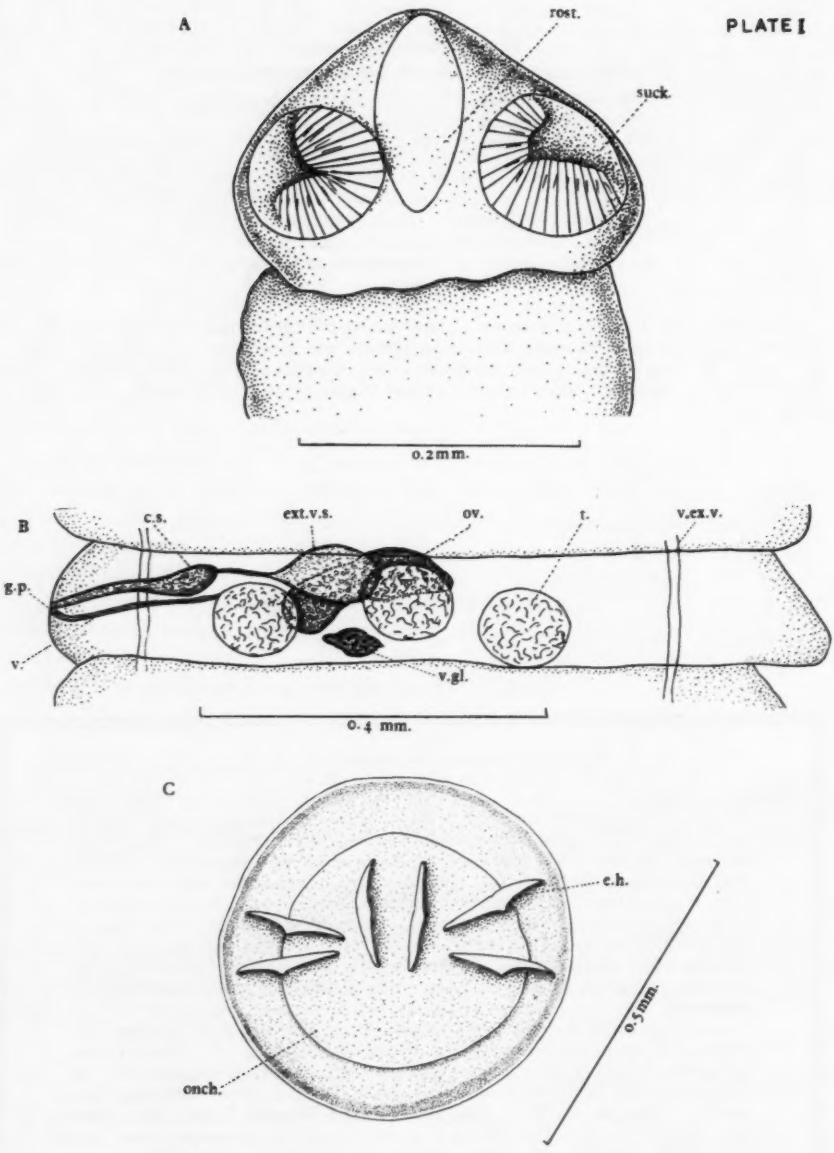
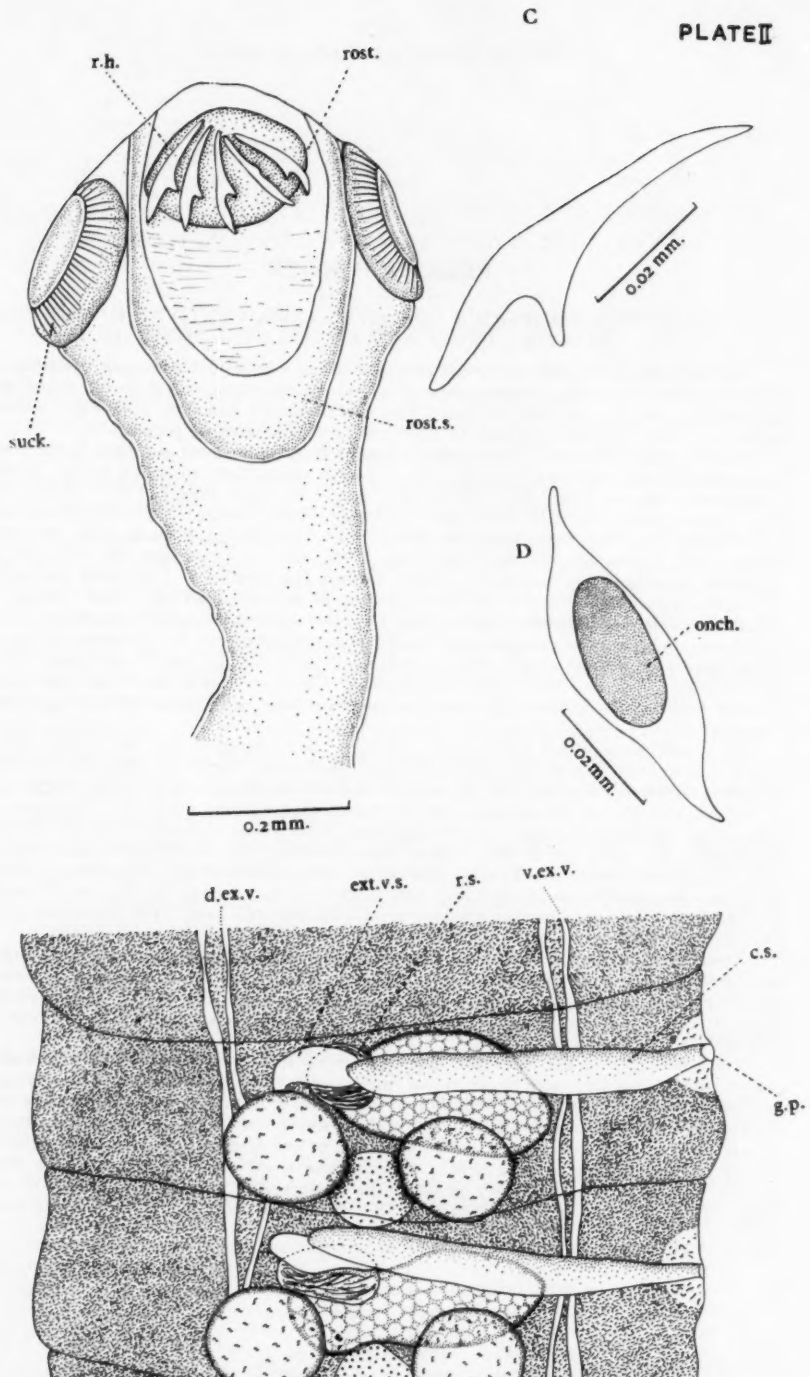


PLATE II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RESEARCH NOTE

MODIFIED TECHNIQUE FOR LONG-TERM CULTIVATION OF *TRYPANOSOMA CRUZI* AND *LEISHMANIA DONOVANI.*

The purpose of this note is to describe a modified semisolid blood agar procedure that permits the hemoflagellates *Trypanosoma cruzi* and *Leishmania donovani* to be maintained in pure culture over long periods of time. This study resulted from the need for a simplified method to provide adequate numbers of these organisms for teaching purposes.

The principal difficulty in maintaining vigorous cultures of these parasites is the need to transfer them frequently, usually at intervals of about 2 weeks, from old to new media. Different investigators, such as Polge (1957, Trans. Roy Soc. Trop. Med. Hyg. **51:** 519), Rubio (1957, Bol. Chileno Parasit. **11:** 62), Citri and Grossowicz (1954, Nature **173:** 1100), Neujean and Evens (1952, Bull. Soc. Path. Exot. **45:** 503), Tobie et al (1950, J. Parasit. Lanc. **36:** 48–54), Chang (1947, J. Inf. Dis. **80:** 164) and Tom (1943, Am. J. Trop. Med. **23:** 615–616) have solved this requirement by techniques suitable to their investigations. Tom notes that cultures of *T. cruzi* remained viable for at least 6 months in rubber-capped test tubes. Chang concluded that a semisolid culture medium was more favorable to the development of *T. cruzi* and species of *Leishmania* than one with a solid base and a fluid overlay, although the presence of agar may interfere with certain types of studies. Pinto (1954, An. Inst. Trop. Lisboa **11:** 573) and Neujean and Evens observed that a combination of physiological saline and agar resulted in better development of these organisms than agar combined with either Tyrode's or Ringer's solutions.

The modified semisolid blood agar (Wenyon, 1926, Protozoology, vol. 2, p. 1299) is prepared as follows: 2 g of Bacto-agar and 8.5 g of sodium chloride are dissolved in 1,000 ml of boiling distilled water and 100 ml aliquots poured into 8-ounce prescription bottles. The bottles are sterilized in the autoclave at 15 pounds pressure and 118 C for 15 minutes, and then cooled for use or storage. For the final step, 10 to 20 ml of sterile whole rabbit's blood are added to the warm (45 to 50 C) 100 ml base solution. The blood is thoroughly mixed to give a uniform suspension. Ten ml aliquots of this suspension are immediately placed in sterile screw-capped test tubes and each tube is checked for sterility at 37 C for 24 hours in the incubator. Only sterile tubes are inoculated with the organisms. The inoculated cultures are stored at room temperature (about 25 C).

About 75 percent of the pure cultures of either species of hemoflagellate will survive in an apparently unchanged state for a period of at least 9 to 11 months; a culture of *L. donovani* has survived for 30 months without addition of any fresh media. The viability of the organisms in the 30-month culture was determined by adding to the old, nearly solid medium about 5 ml of freshly-prepared medium.

The advantages of the procedure described derive from its simplicity: the materials are easily available, defibrinated blood is not used, screw-capped test tubes effectively reduce evaporation of water yet permit sufficient oxygen to leak through without greatly increasing the chances of contaminating the cultures, and no special conditions of storage are required. Consequently, though this type of organism should be transferred every 2 to 3 months when cultured as above, there is little danger of losing the stock colonies if limited time and the pressure of work make it necessary to subculture at infrequent, irregular intervals.—W. PETER HOREN, *Department of Medicine, University of California School of Medicine, San Francisco.*

ON ACANTHOCEPHALA OF TURTLES, WITH THE DESCRIPTION OF *NEOECHINORHYNCHUS EMYDITOIDES* N. SP.

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Acanthocephalans of the genus *Neoechinorhynchus* are common parasites of turtles in the United States and have been found many times since Leidy (1851) reported the first species, *N. emydis*. That more than one species had been confused as *N. emydis* was pointed out by Cable and Hopp (1954) who described two additional ones, *N. pseudemydis* and *N. chrysemydis*, and suggested that still other unrecognized species of *Neoechinorhynchus* may parasitize turtles.

During a routine examination of turtles obtained from a commercial supplier for other purposes, a new acanthocephalan was found and reported in a preliminary note (Cable and Fisher, 1957). That species is described more fully below. In an effort to determine whether still others may occur in those hosts, an extensive study of material from three sources was made: (1) living turtles obtained from various localities, (2) acanthocephala deposited in the Helminthological Collection of the U. S. National Museum, and (3) specimens loaned by individuals. Although no further species were found, the four recognized ones were abundantly represented as a basis for describing the new species and supplementing existing descriptions of the other three. Considerable information bearing on their geographical distribution and host specificity also was obtained.

The study was made under the direction of Prof. R. M. Cable. Mr. Allen McIntosh placed at the author's disposal material in the Helminthological Collection of the U. S. National Museum, and additional specimens were kindly provided by Dr. Margarita Bravo-Hollis of the Instituto de Biologia, Mexico City, and by Dr. Franklin Sogandares-Bernal, Tulane University.

METHODS

Turtles were decapitated and examined soon after being obtained. Those not trapped locally by the author were freshly collected by dealers who supplied also information concerning localities.

Well-extended whole mounts of acanthocephala are difficult to prepare because of the tendency of the proboscis to withdraw and the resistance of the cuticle to the passage of fluids, causing preparations to collapse or become opaque when transferred to mounting resins. Extended specimens with the proboscis and neck fully everted were obtained by two methods. In the first, the worms were placed in tap water in a refrigerator until they were turgid and the everted proboscis was not retracted when touched with a needle. The second method, which was as satisfactory and much more rapid, employed as a relaxing fluid the mixture of menthol, Tween 80, and water recommended by Bailenger and Neuzil (1953), as applied to acanthocephala by Montreuil (1958a).

Some worms were fixed in Bouin's fluid and washed in several changes of 70 percent alcohol containing lithium carbonate to remove the picric acid. Others were fixed in Demke's AFA, and transferred directly to 70 percent alcohol. The glycerine content of that fixative cleared the body wall to the degree that some internal features could be determined without further processing (Montreuil, 1958a). Specimens to be prepared as whole mounts were pricked to

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permit passage of fluids, hydrated, washed with several changes of distilled water, and stained with hematein or Harris' hematoxylin. Following differentiation, they were dehydrated with 2 changes of 95 percent and 2 of absolute alcohol, and gradually cleared by successive treatment for several hours in each of the following alcohol-methyl salicylate mixtures: 3:1, 1:1, and 1:3. To minimize turning opaque when mounted in xylo-damar, the cleared specimens were placed successively in terpineol-damar mixtures in the same ratios as those of alcohol-methyl salicylate above, leaving the material in each mixture for at least 4 hours, a modification of the method described by Montreuil (1958b).

Specimens collected by other investigators had been preserved in divers fluids and varied greatly in the degree of contraction and other conditions affecting the quality of preparations. Permission was granted to make whole mounts of such material. Some of it was very brittle, and was first treated with Demke's fluid, in which it became more pliable, and then handled as described above for fresh material.

The most convenient and sometimes the decisive basis for determining species is the structure of their eggs which cannot be observed satisfactorily in whole mounts. Furthermore, those not completely formed and/or from preserved material can be confusing and misleading. It is generally believed that the selector apparatus permits only fully formed eggs to escape from the female and, for that reason, eggs from the hosts' feces were compared with those obtained by puncture of living females from turtles examined by the author. In no case, were incompletely formed eggs found in the feces, but it was observed that the female may attain almost the full size of her species before containing fully developed ones. It was found also that the outer shell membrane may become inflated and more distinct if living eggs are mounted in water rather than saline.

Adult morphology is shown by drawings made by microprojection. All measurements are in millimeters and those of adult specimens are from whole mounts of fully extended specimens. Lengths of the trunk and external praesoma (i.e., fully extended proboscis and neck less the proboscis receptacle and lemnisci) are stated separately rather than combined in over-all or "body" lengths. Trunk widths were measured at the level of the first ventral nucleus of the subcuticula. The method of determining hook lengths is shown in figure 1 in which parallel

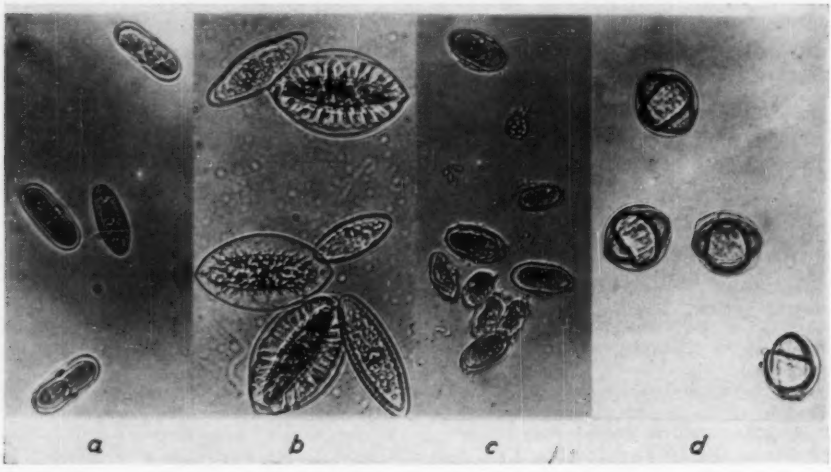


FIGURE 1. Photomicrographs of eggs of (a) *Neoechinorhynchus emyditoides*, living; (b) *N. pseudemydis*, living; (c) *N. emydis*, from preserved material; (d) same, living. All to same scale.

lines are drawn from the base and point of hooks in each circlet as shown. The distance between those lines is given as the length of the hook concerned.

Species deposited in the Helminthological Collection of the U. S. National Museum were identified as indicated by accession numbers following sources of material other than that collected by the author.

DESCRIPTION OF SPECIES

Cable and Hopp (1954) pointed out that an important diagnostic feature of the neoechinorhynchids of turtles is the contour of the posterior end of the female. They reported it to be bilobed in *N. pseudemydis*, but the author has found that a more or less evident cleft occurs at the posterior end of the female of all species. It is accentuated in *N. pseudemydis* by the tapered shape of that end and is deeper than in the other species in which the lobes are superimposed in side view and easily overlooked. Nevertheless, in mixed infections, the author was able to distinguish living females of 3 of the 4 species by the contour of the posterior end alone as verified by subsequent examination of eggs obtained by puncture of the worms.

Neoechinorhynchus emyditoides n. sp.
(Fig. 1a; pls. I and II, figs. 4–7, 18, 22)

Synonym: *Neoechinorhynchus emydis* of Bravo-Hollis, 1946, *nec* Leidy, 1851

Diagnosis: with characters of genus. Body curved ventrally; trunk of female to 34.3 long and 0.940 wide, male to 23.1 long and 0.842 wide. External praesoma 0.207 to 0.255 long, 0.158 to 0.288 wide. Proboscis hooks in 3 rows of 6 hooks each, arranged quincunxially. Lateral hooks of anterior circlet posterior to others, 0.078 to 0.108 long; laterodorsal and lateroventral hooks of that circlet 0.071 to 0.100 long. Hooks of middle circlet similar and in line, measuring 0.045 to 0.061 long. Lateral hooks of basal circlet 0.022 to 0.033 long, others 0.034 to 0.058. Proboscis receptacle 0.420 to 0.750 long, 0.152 to 0.203 wide. Testes contiguous, male reproductive system occupying posterior 42–49 percent of trunk length; anterior testis 1.386 to 1.504 long, 0.380 to 0.472 wide; posterior testis 1.118 to 1.386 by 0.311 to 0.483, immediately followed by cement gland measuring 1.45 to 1.80 long, 0.248 to 0.300 wide. Cement receptacle globular, posterior to cement gland, its duct extending posteriorly to open in bursa. Mouth of uterine bell 0.552 to 1.14 from posterior end of female; uterus exclusive of bell and selector apparatus 0.175 to 0.375 long, 0.083 to 0.158 wide; vagina 0.135 to 0.203 long, 0.053 to 0.068 wide. Posterior end of female usually rounded, but in well extended specimens sometimes with a slightly irregular contour very similar to that of *N. emydis*; genital pore in posterior furrow, slightly ventral to long axis of trunk. Eggs 0.025 to 0.030 long, 0.010 to 0.013 wide; middle membrane slightly inflated at poles, usually with 4 to 6 short, tube-like structures not at ends of egg shell. Outer membrane thin, usually distinct except at poles where closely applied to middle membrane, best demonstrated by placing living eggs in water and permitting membrane to swell. Acanthor 0.022 to 0.025 by 0.005 to 0.006.

Hosts and localities:

A. Material collected by author:
Pseudemys scripta elegans (Wied), Arkansas, Mississippi. *nec. P. s. troosti* as given in Cable and Fisher, 1957.

B. Other material and sources:
Pseudemys s. elegans, Texas (27254), N. Y. Aquarium (36626), "California Zoo" (42739), Virginia (44210).
Emys blandingii, Massachusetts (34570). "Hard-shell turtle," California (47043).
Chrysemys ornata, Mexico (Bravo-Hollis).

Type specimens: Holotype (female) and paratype (male). No. 39025, Helminthological Coll., U. S. National Museum, from *Pseudemys scripta elegans* from St. Francis River, Arkansas.

Neoechinorhynchus emyditoides is so named because it resembles *N. emydis* in the contour of the posterior end of the female and small egg size but differs from that species in the size attained by both sexes, width of the uterus, and characteristics of the eggs.

Several investigators have reported a fertilization cap that is formed during copulation by the cement gland secretion of the male acanthocephalan and fits over the posterior end of the female. Its function, according to Hyman (1951), is to plug the genital pore and thus prevent the escape of sperms. The author found

numerous females with fertilization caps, usually light green in color and, presumably, stained by bile pigments. Figure 22 of plate II shows a sagittal section of the posterior end of a female *N. emyditoides* bearing such a cap. Besides many eggs next to the posterior end of the worm, it contains numerous sperms evidently pushed away from that region by the eggs and pressed against the fertilization cap. It thus seems that the cap may serve more as a spermatophore than a plug to retain sperms that have gained entry to the female. Otherwise, one would expect that although both eggs and sperms may be discharged, the two would be mixed together and not in the distinct zones observed. The accumulation of eggs in the cap probably ruptures it or forces it from the end of the female.

Cable and Hopp (1954) described *Neoechinorhynchus pseudemydis* from *Pseudemys s. elegans* in northwestern Indiana. In the present study, the species has been found in other hosts and localities which are included in the following description. It extends that given by Cable and Hopp, particularly with respect to the eggs which they characterized from specimens preserved in alcohol.

Neoechinorhynchus pseudemydis Cable and Hopp, 1954
(Fig. 1b; pls. I and II, figs. 1–3, 21)

With characters of genus. Body very long and slender, curved ventrally but not strongly so. Females to 39.2 long, 1.25 wide; males to 26.4 long, 0.89 wide. External praesoma 0.148 to 0.189 long, 0.198 to 0.240 wide. Proboscis hooks arranged as in preceding species. Lateral hooks of anterior circlet 0.085 to 0.109 long, others of that circlet 0.067 to 0.096. Hooks of middle circlet similar in size, 0.050 to 0.060 long. Lateral hooks of basal circlet 0.028 to 0.034 long, others 0.042 to 0.050. Testes arranged as in preceding species, male reproductive system occupying 35 to 42 percent of trunk length. Anterior testis 1.85 to 2.14 long, 0.40 to 0.44 wide; posterior testis 2.60 to 2.90 by 0.43 to 0.45. Cement gland 1.65 to 3.00 long, 0.255 to 0.375 wide; cement receptacle and duct as in preceding species. Mouth of uterine bell 0.625 to 1.275 from posterior end of female; uterus exclusive of bell and selector apparatus 0.233 to 0.360 long, 0.100 to 0.157 wide; vagina 0.146 to 0.232 by 0.058 to 0.069. Posterior end of female tapers from about level of uterus to terminate in 2 lateral, somewhat swollen lobes with genital pore in furrow between them. Living eggs broadly spindle-shaped, 0.042 to 0.054 long, 0.018 to 0.028 wide; acanthor 0.033 to 0.038 by 0.009 to 0.011; middle shell membrane with numerous uniformly distributed tube-like structures; outer shell thin, swelling in water and easily lost.

Hosts and localities:

A. Material collected by author:
Pseudemys scripta elegans (Wied), Indiana, Arkansas, Mississippi.
Chrysemys picta marginata (Agassiz), Indiana.
Emys blandingii (Holbrook), Southern Minnesota.

B. Other material and sources:
Pseudemys s. elegans, Missouri (18866), Texas (27254), Massachusetts (38680), Virginia (44210), Indiana (49100), Illinois (50945).
Pseudemys s. troosti, Illinois (50951).
"mud turtle," Illinois (50947).

Cable and Hopp (1954) described *Neoechinorhynchus chrysemydis* from 2 specimens, a mature male and female, from *Chrysemys picta marginata* in northwestern Indiana. To obtain eggs for study, they found it necessary to puncture the female which, as a result, contracted at the posterior end. Additional material obtained by the author has confirmed the validity of the species and serves as a basis for the following description which extends the necessarily limited one given by Cable and Hopp:

Neoechinorhynchus chrysemydis Cable and Hopp, 1954
(Pls. I and II, figs. 14–17, 23)

With characters of genus. Body moderately long and slender, with slight ventral curvature. Females to 15.34 long, 0.73 wide; males to 12.9 by 0.69. External praesoma 0.132 to 0.233 long,

0.200 to 0.210 wide. Proboscis hooks arranged as in preceding species. Lateral hooks of anterior circlet 0.08 to 0.14 long, other hooks of that circlet 0.048 to 0.096. Hooks of middle circlet similar, 0.044 to 0.061 in length; lateral hooks of basal circlet 0.021 to 0.037 long, others of that circlet 0.038 to 0.053. Male reproductive system occupies 45 to 51 percent of trunk length. Anterior testis 0.645 to 0.980 long, 0.190 to 0.263 wide; posterior testis 0.638 to 0.810 by 0.190 to 0.278. Cement gland 1.13 to 1.22 long, 0.263 to 0.360 wide. Mouth of uterine bell 0.69 to 0.84 from posterior end of female; uterus exclusive of bell and selector apparatus 0.199 to 0.248 long, 0.058 to 0.096 wide; vagina 0.136 to 0.210 by 0.050 to 0.062. Posterior end of female with a distinct papilla at ventral end of furrow between short lobes; genital pore ventral, slightly anterior to papilla. Eggs elongate, curved in "lateral" aspect, 0.055 to 0.060 long, 0.019 to 0.022 wide; middle membrane thickened at ends of egg where several small tube-like structures are present. Acanthor 0.028 to 0.030 by 0.008 to 0.009.

Hosts and localities:

A. Material collected by author:
Pseudemys scripta elegans (Wied), Arkansas.

B. Other material and sources:
Chrysemys picta marginata, Indiana (49099).
"Southern painted turtle," Minnesota(?) (34536).
Pseudemys scripta subsp., Louisiana (Sogandares-Bernal).

The caudal papilla of the female is a consistent diagnostic feature of *N. chrysemydis* and, as Cable and Hopp (1954) surmised, the species is more common in the South and is rare as far north as northwestern Indiana.

Hopp (1954) gave an adequate description of *Neoechinorhynchus emydis* in his study of the life history. He found that species only in *Graptemys geographica*, but the present study has revealed that it may occur also in other turtles. The following redescription includes the new host data and extends somewhat other limits that Hopp placed on the species.

Neoechinorhynchus emydis (Leidy, 1851)
(Figs. 1c, 1d; pls. I and II, figs. 8–10, 11–13, 19–20)
Syn. *Echinorhynchus hamulatus* Leidy, 1856

With characters of genus. Body rather short and thick, curved ventrally. Trunk of females to 22.2 long, 1.5 wide; of males to 14.5 by 1.04. External praesoma 0.175 to 0.248 long, 0.216 to 0.300 wide. Hook arrangement as in preceding species. Lateral hooks of anterior circlet 0.091 to 0.107 long, others of that circlet 0.069 to 0.080. Hooks of middle circlet similar, 0.048 to 0.060 long. Lateral hooks of basal circlet 0.033 to 0.046 long, others of that group 0.038 to 0.050. Proboscis receptacle 0.532 to 0.733 long, 0.185 to 0.192 wide. Male reproductive system occupies 42 to 50 percent of trunk length. Anterior testis 0.9 to 1.4 long, 0.293 to 0.330 wide; posterior testis 0.88 to 0.93 by 0.314 to 0.362; cement gland 1.34 to 1.76 long, 0.225 to 0.307 wide. Mouth of uterine bell 0.833 to 1.08 from posterior end of female; uterus exclusive of bell and selector apparatus 0.277 to 0.360 long, 0.046 to 0.091 wide; vagina 0.172 to 0.263 by 0.043 to 0.060. Posterior end of female rounded to irregular and somewhat pointed, genital pore toward ventral end of furrow between lobes. Living eggs 0.023 to 0.025 by 0.015 to 0.019; middle shell membrane with the appearance of a conspicuous C-shaped vacuole extending partly around egg at equator; tubular structures absent. Acanthor 0.019 to 0.024 by 0.009 to 0.013.

Hosts and localities:

A. Material collected by author:
Graptemys geographica (LeSueur), northwestern Indiana.
Emys blandingii (Holbrook), southern Minnesota.

B. Other material and sources:
Graptemys geographica, Indiana (37406, 37407).
"painted turtle," N. Y. Aquarium (34511).
Graptemys pseudogeographica (Gray), Illinois (50946).
Pseudemys scripta subsp., locality unknown, (50954).

Figure 1c and plates I and II, figures 8 to 10 and 19 are from material that at first seemed to be of an additional species of *Neoechinorhynchus* and consisted of many specimens taken from *Graptemys pseudogeographica*, preserved in alcohol, and deposited in the U. S. National Museum over 40 years ago. All of the worms

were contracted and the eggs seemed to be different from those of any of the above species. However, a very few eggs were found that were typical of *N. emydis* and the appearance of the others may be attributed to their being incompletely formed and to long preservation. The difficulty of identifying this material emphasizes the importance of fully mature specimens and their preservation fully extended.

DISTRIBUTION AND HOST SPECIFICITY

Data concerning the distribution of neoechinorhynchids of turtles are incomplete and some of the localities given above are unreliable because turtles often are shipped considerable distances to laboratories and zoos. It seems evident, however, that *N. emydis* is the species most restricted in its distribution, occurring from New England westward to the Mississippi system and as far south as Kentucky where it presumably was the species with which Lincicome (1948) was concerned. In contrast to *N. emydis, N. chrysemydis,* and *N. emyditoides* are more southern in distribution. The known range of *N. chrysemydis* is from northwestern Indiana, where it is very rare, to Louisiana, and that of *N. emyditoides* is from Arkansas to Mexico; their east-west range is less certain. Perhaps most widely distributed of the 4 is *N. pseudemydis* which is known to occur from Massachusetts westward to Minnesota and southward to Louisiana.

The species of turtles examined by the author and the parasites obtained are included in table I from which it is evident that host specificity is by no means as

TABLE I. *Turtles examined and their species of* Neoechinorhynchus.

Species	Locality	No. examined	No. positive	Parasites obtained
Pseudemys s. elegans	Arkansas	24	17	*N. pseudemydis* *N. emyditoides* *N. chrysemydis*
	Louisiana	12	6	*N. pseudemydis* *N. emyditoides*
	Mississippi	12	12	*N. pseudemydis* *N. emyditoides*
	Indiana	1	1	*N. pseudemydis*
Chrysemys p. marginata	Indiana	102	1	*N. pseudemydis*
	Minnesota	6	0	
Chelydra serpentina	Indiana	26	1	*N. chrysemydis* (immature)
Amyda ferox muticus	Indiana	3	0	
Emys blandingii	Minnesota	6	3	*N. pseudemydis* *N. emydis*
	Indiana	2	0	
Graptemys geographica	Indiana	1	1	*N. emydis*
Terrapene c. carolina	Indiana	2	0	

rigid as Cable and Hopp (1954) believed. The present data, however, confirm their distinction of species as opposed to the opinion of Van Cleave and Bullock (1950) that turtles harbor but 1 species in North America. The finding of 3 of the 4 species not only in a single host species, but even in an individual of that species, removes any possibility that differences here considered to be specific ones may be attributed to variation in a single species associated with development in different hosts.

Parasitism of turtles by acanthocephala seems to depend more on ecology and geographic distribution of the parasites and the hosts' food habits than on host specificity. The rare occurrence of *N. emydis* in turtles other than *Graptemys geographica* may be due to that turtle's feeding primarily on mollusks which harbor juveniles of *N. emydis* and having jaws modified for cracking shells, whereas other turtles are not so adapted. However, they may feed to a limited extent on mollusks,

especially young ones or small species, and thereby occasionally become infected with *N. emydis*. The necessity of a mollusk in the life history of *N. emydis* has not been established experimentally, but it seems certain that *G. geographica* acquires that parasite largely if not exclusively by eating snails rather than the ostracod intermediate host. According to Ward (1940), a similar situation obtains in the life history of *N. cylindratus* in which the definitive host, a bass, seems to obtain most if not all of the parasites by ingesting another fish rather than the ostracod host. Except for *N. emydis*, however, the life histories of neoechinorhynchids in turtles are still unknown and until that information is available the question of food habits versus host specificity cannot be fully answered.

SUMMARY

A survey of acanthocephala from North American turtles included the examination of 197 turtles representing 7 species from the Mississippi drainage system and the study of material deposited in the U. S. National Museum and specimens loaned by individuals. Descriptions and figures are given for the 3 previously reported species and *Neoechinorhynchus emyditoides* n. sp. which is readily identified by the eggs. The discovery of 3 of the 4 species in a single host confirms their validity and, with other observations, indicates that the species harbored by turtles may depend less on host specificity than on the ecology and distribution of the parasites and the feeding habits of potential hosts.

REFERENCES CITED

BAILENGER, J. AND NEUZIL, E. 1953 Nouvelles techniques d'examen des helminthes: Fixation; coloration; montage. Ann. Parasit. **28:** 392–398.

BRAVO-HOLLIS, M. 1946 *Neoechinorhynchus emydis* (Leidy, 1852); Van Cleave, 1913, parasito del intestino de *Chrysemys ornata*. An. Inst. Biol. Nac. México **17:** 187–192.

CABLE, R. M. AND HOPP, W. B. 1954 Acanthocephalan parasites of the genus *Neoechinorhynchus* in North American turtles with the description of two new species. J. Parasit. **40:** 674–680.

——— AND FISHER, F. M., JR. 1957 A fourth species of *Neoechinorhynchus* (Acanthocephala) in turtles of the United States. J. Parasit. **43:** (supp.): 29.

HOPP, W. B. 1954 Studies on the morphology and life cycle of *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of the map turtle, *Graptemys geographica* (Le Sueur). J. Parasit. **40:** 284–299.

HYMAN, L. H. 1951 The Invertebrates. Vol. 3, New York.

LEIDY, J. 1851 Contributions to helminthology. Proc. Acad. Nat. Sci. Phila. **5:** 205–209.

LINCICOME, D. R. 1948 Observations on *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of turtles. J. Parasit. **34:** 51–54.

MONTREUIL, P. L. 1958a Relaxation and fixation of Acanthocephala. Can. J. Zool. **36:** 263–264.

——— 1958b *Corynosoma magdaleni*, sp. nov. (Acanthocephala), a parasite of the Gray Seal in Eastern Canada. Can. J. Zool. **36:** 205–215.

VAN CLEAVE, H. J. AND BULLOCK, W. L. 1950 Morphology of *Neoechinorhynchus emydis*, a typical representative of the Eoacanthocephala. I. The praesoma. Tr. Am. Micr. Soc. **69:** 288–308.

WARD, H. L. 1940 Studies on the life history of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) (Acanthocephala). Tr. Am. Micr. Soc. **59:** 327–347.

Explanation of Plates

Abbreviations: EG, egg or shelled embryo; OB, ovarian ball; SA, selector apparatus; SP, sperm; UB, uterine bell; UT, uterus; VA, vagina.

Plate I

(Corresponding figures of all species drawn to the same scale)

Figure 1. *Neoechinorhynchus pseudemydis,* lateral view of anterior end.
Figure 2. Same, end view of posterior end of female.
Figure 3. Same, living egg or shelled embryo.
Figure 4. *Neoechinorhynchus emyditoides,* lateral view of anterior end.
Figure 5. Same, end view of posterior end of female.
Figures 6, 7. Same, living eggs.
Figure 8. *Neoechinorhynchus emydis,* lateral view of anterior end, specimen long preserved, not well extended.
Figure 9. Same species and material, end view of posterior end of female.
Figure 10. Same species and material, eggs.
Figure 11. Same species, lateral view of anterior end, well extended.
Figure 12. Same, end view of posterior end of female.
Figure 13. Same, living eggs.
Figure 14. *Neoechinorhynchus chrysemydis,* lateral view of anterior end.
Figure 15. Same, end view of posterior end of female.
Figures 16, 17. Same, living eggs.

Plate II

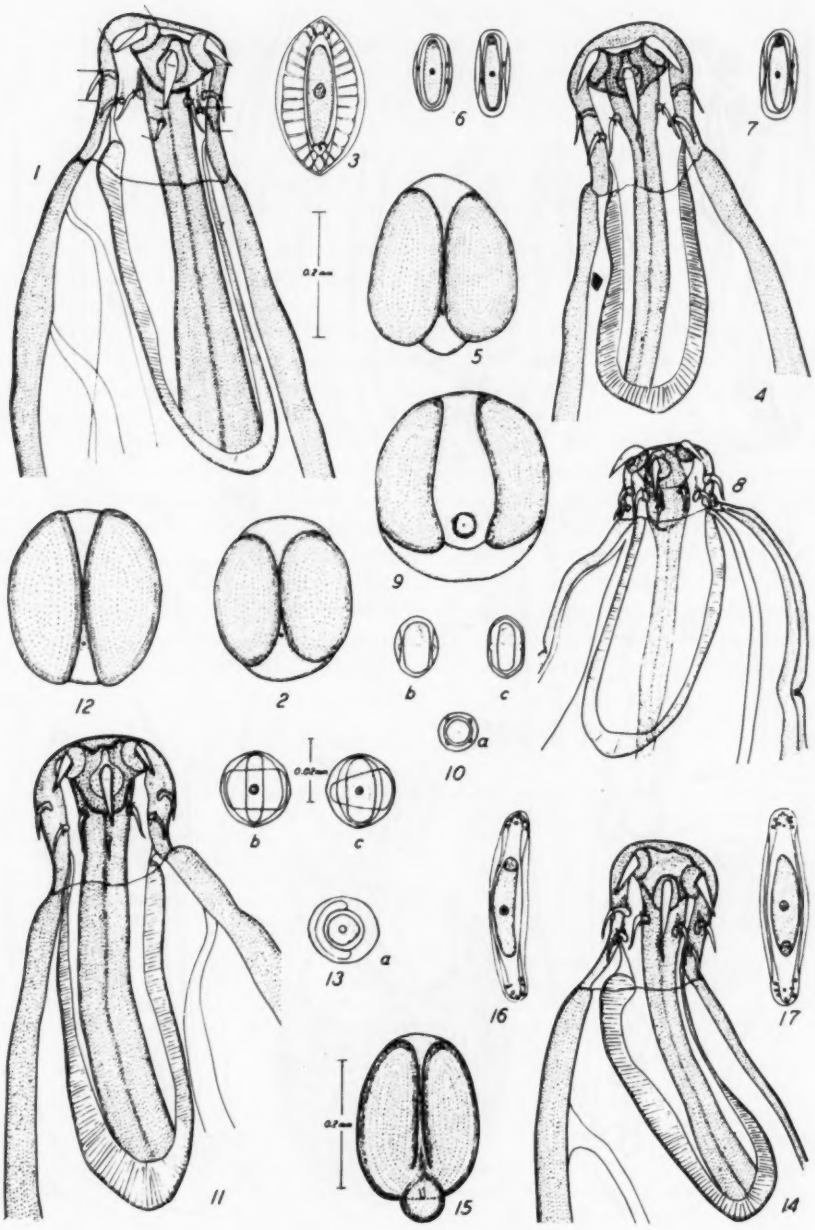
(Posterior ends of females, drawn to same scale)

Figure 18. *Neoechinorhynchus emyditoides,* side view,
Figure 19. *Neoechinorhynchus emydis,* contracted from long preserved material.
Figure 20. Same species, well extended.
Figure 21. *Neoechinorhynchus pseudemydis.*
Figure 22. *Neoechinorhynchus emyditoides,* sagittal section through posterior end and fertilization cap.
Figure 23. *Neoechinorhynchus chrysemydis.*

ERRATUM

Journal of Parasitology, vol. **45,** 390, paragraph 2, line 4 should read, ". . . the effects of endocrine substances of vertebrates on an invertebrate species."—Horace W. Stunkard.

PLATE I



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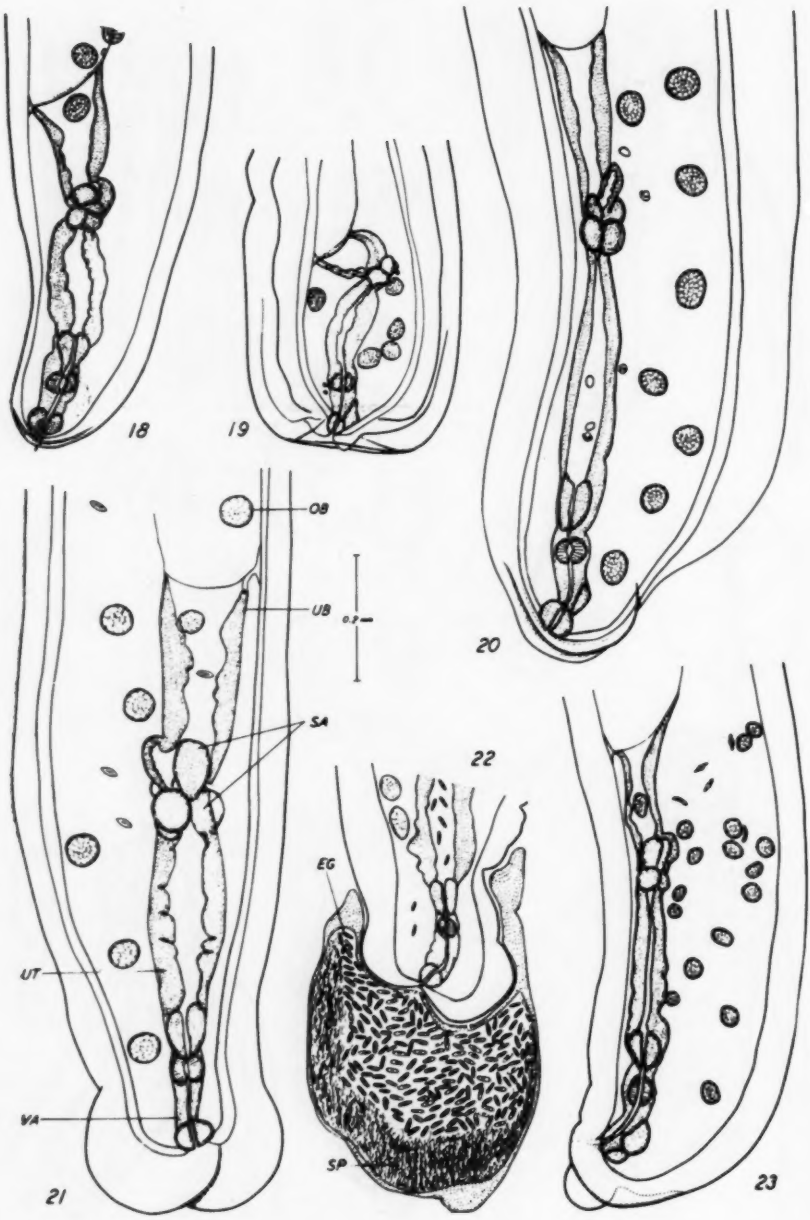

THE ANTHELMINTIC ACTION OF PHENOTHIAZINE AND PIPERAZINE AGAINST *HETERAKIS GALLINAE* AND *ASCARIDIA GALLI* IN CHICKENS

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Although phenothiazine and piperazine are anthelmintics commonly used in poultry, reports differ as to their value against the common poultry parasites, *Heterakis gallinae* and *Ascaridia galli.*

Roberts (1940) and Allen, Olivier, and Peterson (1942) indicated that phenothiazine showed anthelmintic action against both *Heterakis* and *Ascaridia;* on the other hand, Guilhon (1948), Martin Vaquero (1952), and others found that phenothiazine had little, if any, effect against *Ascaridia.*

Sloan, Kingsbury, and Jolly (1954), Shumard and Eveleth (1955), Shumard (1956), and others found that piperazine exhibited effective action against *Ascaridia* in poultry. Shumard (1957) found also that piperazine exhibited some anthelmintic action against *Heterakis.* Edgar, Davis, and Frazier (1957) obtained erratic results against *Heterakis* with piperazine compounds in water, feed, tablet, and capsule, although they felt that piperazine was about as effective as phenothiazine. Polakova (1957), using piperazine adipate, reported an efficacy of 23 percent against *Heterakis.*

Because of these conflicting reports, trials were undertaken to determine the effectiveness of phenothiazine, piperazine, and mixtures of the two compounds against *Heterakis* and *Ascaridia* in chickens.

MATERIALS AND METHODS

Seventy-two mature White Leghorn, Barred Plymouth Rock, and Rhode Island Red chickens were used in a series of 5 experiments. The birds harbored naturally acquired infections of one or more helminths commonly found in this locality, namely, *Heterakis gallinae, Ascaridia galli, Capillaria* spp., *Hymenolepis* spp., and *Raillietina cesticillus.*

The birds were confined in individual cages with raised wire floors through which droppings passed to removable metal trays. Each cage was equipped with separate feed and water containers, which were attached to the outside of the cages. Feces were collected and examined for parasites daily. Complete worm counts were made when the birds were necropsied 4 days after treatment was initiated.

Water was withheld for about 18 hours from all birds that were given piperazine in drinking water; but in all other trials, feed and water were available at all times. The citrate salt was used and all doses were calculated in terms of piperazine base. The phenothiazine, given by capsule or in feed, was a National Formulary grade powder with an average particle size of about 10 microns.

EXPERIMENTAL DATA

The results are summarized in table I.

Experiment 1. Phenothiazine by capsule. Each of 6 birds, weighing from 2.2 to 3.9 kg, was given 0.5 g of phenothiazine by capsule. Although this is the dosage commonly recommended for removing cecal worms from chickens, only 39 percent (128) of 326 *Heterakis* were eliminated from the 6 birds; 21 percent (4) of 19 *Ascaridia* were removed from 2.

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One-gram doses of phenothiazine were given by capsule to each of 6 birds weighing from 1.9 to 3.1 kg. In these trials, the efficiency against cecal worms was substantially increased, the chemical removing 94 percent (865) of 913 *Heterakis* from 5 birds; 24 percent (5) of 21 *Ascaridia* were removed from 2. Although the data are limited, the findings suggest that more than 0.5 g of phenothiazine may be necessary to obtain satisfactory efficiency against cecal worms.

TABLE I. *Efficacy of phenothiazine and piperazine citrate against* Heterakis gallinae *and* Ascaridia galli *in chickens.*

Experiment No.	Treatment		Parasites			Efficacy (pct.)	Trials (no.)
	Chemical	Regimen	Name	Removed (no.)	Left (no.)		
1	phenothiazine	0.5 g (c)	*Heterakis*	128	198	39	6
			Ascaridia	4	15	21	2
			Capillaria	0	74	0	3
		1.0 g (c)	*Heterakis*	865	48	94	5
			Ascaridia	5	16	24	2
			Capillaria	0	393	0	4
2	phenothiazine	0.74 pct. (f)	*Heterakis*	188	29	86	6
			Ascaridia	23	55	29	6
			Capillaria	0	11	0	2
3	piperazine citrate	100 mg* (c)	*Heterakis*	343	408	45	11
			Ascaridia	114	0	100	9
			Capillaria	11	20	35	7
			Hymenolepis	0	155	0	5
			Raillietina	0	13	0	1
		200 mg* (c)	*Heterakis*	1089	555	66	11
			Ascaridia	22	0	100	4
			Capillaria	1227	2949	29	9
			Hymenolepis	0	46	0	2
4	piperazine citrate	0.05 pct. (w)	*Heterakis*	319	365	45	8
			Ascaridia	740	8	99	6
			Capillaria	7	37	3	6
			Hymenolepis	0	203	0	2
		0.1 pct. (w)	*Heterakis*	222	926	19	13
			Ascaridia	23	1	96	4
			Capillaria	3	90	3	9
			Hymenolepis	0	58	0	2
5	phenothiazine and piperazine citrate	600 mg 50 mg* (c)	*Heterakis*	615	55	92	6
			Ascaridia	17	0	100	2
	phenothiazine and piperazine citrate	700 mg 100 mg* (c)	*Heterakis*	723	45	94	6
			Ascaridia	91	0	100	3
			Capillaria	80	11	88	3

(c), capsule; (f), in feed for 3 days; (w), in drinking water for 24 hours.
* Piperazine base.

Experiment 2. Phenothiazine in feed. Each of 6 birds, weighing from 2.5 to 4.0 kg, was given feed containing 0.74 percent phenothiazine ad libitum for 3 days. The birds consumed an average of 3.2 g (2.38 to 4.44 g) of phenothiazine. The treatment removed 86 percent (188) of 217 *Heterakis* and 29 percent (23) of 78 *Ascaridia* from the 6 birds. Curiously, the aggregate efficacy against *Heterakis* was slightly lower than that obtained with single 1-gram doses, despite the larger amount of phenothiazine per bird.

Experiment 3. Piperazine by capsule. Eleven birds, weighing from 1.9 to 3.9 kg, were each given 100 mg of piperazine by capsule. The treatment removed 45 percent (343) of 751 *Heterakis* from 11 birds and all of 114 *Ascaridia* from 9. A dose of 200 mg, given to each of 11 birds weighing from 1.1 to 3.3 kg, removed 66 percent (1,089) of 1,644 *Heterakis* from the 11 birds and all of 22 *Ascaridia* from 4. The large dose of piperazine resulted in somewhat increased efficiency, but the result was still short of that desired. No anthelmintic action was shown against *Hymenolepis* spp. or *Raillietina* spp.

Experiment 4. Piperazine in water. Piperazine was given in drinking water for 24 hours after withholding water from the birds overnight. At a concentration of 0.05 percent, the chemical removed 45 percent (319) of 684 *Heterakis* from 8 birds and 99 percent (740) of 748 *Ascaridia* from 6. The birds consumed, on the average, 150 mg of piperazine (130 to 200 mg). When the concentration was increased to 0.1 percent, the treatment removed 96 percent (23) of 24 *Ascaridia* from 4 birds but only 19 percent (222) of 1,148 *Heterakis* from 13. The average consumption of piperazine was 240 mg (90 to 450 mg) and attests the unreliable action of the chemical against cecal worms. No anthelmintic action was shown against *Hymenolepis* spp.

Experiment 5. Phenothiazine-piperazine mixtures. Single 0.75-gram doses of a 12:1 mixture of phenothiazine and piperazine were given by capsule to 6 birds weighing from 2.0 to 3.0 kg. In the aggregate, 92 percent (615) of 670 *Heterakis* were removed from 6 birds and all of 17 *Ascaridia* from 2. Similar results were obtained with 1-gram doses of a 7:1 mixture given by capsule to 6 birds weighing from 2.1 to 3.4 kg. In these trials, the treatment removed 94 percent (723) of 768 *Heterakis* from 6 birds and all of 91 *Ascaridia* from 3. The data suggest that appropriate mixtures of phenothiazine and piperazine may be more effective against cecal worms than either drug alone. The 7:1 mixture appeared to be effective against *Capillaria* spp.; but it is emphasized that all 80 specimens eliminated after treatment were recovered from only 1 of 3 infected birds.

DISCUSSION

In these limited trials with chickens, phenothiazine exhibited only minimal action against *Ascaridia,* but it was very effective against *Heterakis.* The data suggest, however, that the dosage level necessary to achieve maximum efficacy against cecal worms is probably above the 0.5 g per bird that is now commonly recommended. The chemical also produced favorable results when used as a feed additive. The aggregate efficacy was slightly lower, however, than that obtained with single 1-gram doses, although the average consumption per bird was slightly more than 1 g daily for 3 successive days. Additional critical data are needed to re-define the optimum dosage for individual treatment and to establish the most feasible regimen for flock use.

Piperazine was effective against *Ascaridia,* but showed no promise of reliable action against *Heterakis.* Moreover, administration of the chemical in drinking water for 24 hours failed to increase efficiency against cecal worms, although the intake in many birds resulted in doses considerably larger than those given by capsule. The amount of piperazine consumed by individual birds had no significant effect on anthelmintic action. One bird that consumed 450 mg of the chemical eliminated only 2 of 111 *Heterakis,* whereas 79 percent of 160 cecal worms were removed from a bird that obtained 410 mg. This unreliable action of piperazine was not correlated with the size of the birds or the degree of infection.

Mixtures of the two drugs produced reliable anthelmintic action against both *Heterakis* and *Ascaridia.* The efficiency against *Heterakis* was similar to that achieved with larger single doses of phenothiazine and suggest a possible augmentative relationship of the two drugs. Additional critical trials are desirable to determine the effectiveness of other mixtures for individual or flock treatment against these parasites.

SUMMARY

Critical anthelmintic trials were made with 72 chickens to ascertain the efficiency of phenothiazine, piperazine citrate, and mixtures of the 2 chemicals against *Heterakis gallinae* and *Ascaridia galli*.

Phenothiazine (N.F. powder, about 10-micron particle size), given in single doses of 0.5 g in hard gelatin capsules, removed 39 percent (128) of 326 *Heterakis* from 6 chickens and 21 percent (4) of 19 *Ascaridia* from 2. One-gram doses removed 94 percent (865) of 913 *Heterakis* from 5 birds and 24 percent (5) of 21 *Ascaridia* from 2. Given at 0.74 percent of the feed for 3 days, the chemical removed 86 percent (188) of 217 *Heterakis* and 29 percent (23) of 78 *Ascaridia* from 6 birds.

Piperazine citrate, given by capsule in single doses containing 100 mg of piperazine, removed 45 percent (343) of 751 *Heterakis* from 11 chickens; and 200-mg doses removed 66 percent (1,089) of 1,644 *Heterakis* from a similar group. Both doses were completely effective against *Ascaridia*. Given in drinking water for 24 hours at 0.05 percent piperazine base, the chemical removed 45 percent (319) of 684 *Heterakis* from 8 birds and 99 percent (740) of 748 *Ascaridia* from 6. The birds consumed an average of 150 mg of piperazine. When the concentration was increased to 0.1 percent, the chemical removed 19 percent (222) of 1,148 *Heterakis* from 13 birds and 96 percent (23) of 24 *Ascaridia* from 4. The birds consumed an average of 240 mg of piperazine, although total water consumption was slightly lower than in the previous group.

Single 1-gram doses of a 7 : 1 mixture of phenothiazine and piperazine removed 94 percent (723) of 768 *Heterakis* from 6 birds and all of 91 *Ascaridia* from 3. Comparable results were obtained against both species with 0.75-gram doses of a 12 : 1 mixture of the two chemicals.

REFERENCES

ALLEN, R. W., OLIVER, L. J. AND PETERSON, H. O. 1942 The efficacy of phenothiazine for the removal of the cecal worm of chickens. Vet. Med. **37:** 412–415.

EDGAR, S. A., DAVIS, D. C. AND FRAZIER, J. A. 1957 The efficacy of some piperazine compounds in the elimination of helminths from experimentally- and naturally-infected poultry. Poul. Sci. **36:** 495–510.

GUILHON, J. 1948 Recherches sur les propriétés anthelmintiques de quelques dérivés de la thiodiphénylamine (Anthelmintic properties of phenothiazine derivatives). Bull. Acad. Vét. France **21:** 227–232.

MARTIN VAQUERO, B. 1952 Ascaridiosis de las gallinas. *Ascaridia galli* fenotiazina y tetracloruro de carbono. Veterinaria, Madrid **16:** 807–814.

POLÁKOVÁ, MILENA 1957 Adipát piperazinu při askaridiose a heterakidose slepic (The treatment of ascaridosis and heterakidosis in hens with piperazine adipate) [Czechoslovakian text; English and German summaries]. Sborn. Českoslov. Akad. Zeměděl. Věd., Vet. Med. **30:** 865–880.

ROBERTS, F. H. S. 1940 A preliminary note on the efficiency of phenothiazine against some poultry helminths. Austral. Vet. J. **16:** 172–174.

SHUMARD, R. F. 1956 The anthelmintic activity of powder and liquid parvex against *Ascaridia galli* and *Heterakis gallinae*. J. Parasit. **42** (sec. 2, supp.) : 13.

———1957 The toxicity to chickens and the anthelmintic effect of two forms of a piperazine-carbon disulfide complex on *Ascaridia galli* and *Heterakis gallinae*. Poul. Sci. **36:** 613–618.

———, AND EVELETH, D. F. 1955 A preliminary report on the anthelmintic action of piperazine citrate on *Ascaridia galli* and *Heterakis gallinae* in hens. Vet. Med. **50:** 203–205.

SLOAN, J. E. N., KINGSBURY, P. A. AND JOLLY, D. W. 1954 Preliminary trials with piperazine adipate as a veterinary anthelmintic. J. Pharm. and Pharmacol. **6:** 718–724.

RESEARCH NOTE

REPLACEMENT OF SERUM IN THE CULTIVATION OF *HISTOMONAS MELEAGRIDIS.*

No entirely satisfactory technique has been devised for propagating, in a "chemically defined medium," the protozoan parasite *Histomonas meleagridis*, the cause of blackhead of poultry. Until now the techniques for culturing this organism have generally involved the use of a basal medium to which serum and bacteria were added.

As a first step towards a possible chemically defined medium, attempts were made to ascertain which of the factors in the blood serum are essential growth factors for *H. meleagridis*. This led to the substitution of various compounds for the serum.

The basal medium used in the investigations reported here consisted of 9 g of NaCl, 0.4 g of KCl, 0.2 g of $CaCl_2$, 0.2 g of dextrose, and 1,000 ml of distilled water. The medium was dispensed in 4.5 ml quantities into 15 ml screw-capped culture tubes and sterilized in an autoclave at 15 pounds pressure for 20 minutes. The tubes were then refrigerated until needed. Just prior to use each tube received 0.5 ml of sterile Seitz-filtered bovine serum (inactivated at 56 C for 30 minutes) or a serum substitute. Each tube also received one ml of a nutrient broth culture containing a mixture of bacteria, originally obtained from the cecal droppings of turkeys. However, before being added, the bacterial culture was subjected for at least 1 hour to the action of penicillin G potassium and dihydrostreptomycin sulphate in the proportions of 7,100 units per ml and 0.05 g per ml of culture, respectively.

One to 2 ml of existing histomonad cultures were then added to each tube. These histomonads were originally isolated from the ceca of experimentally infected chickens. To each of the cultures were then added 1,400 units per ml of penicillin G potassium, 0.01 g per ml of dihydrostreptomycin sulphate, and 125 units per ml of mycostatin. Each culture was then overlaid with 0.5 ml of white petroleum jelly that had been liquefied in a flamed hypodermic syringe. The tubes were then "capped" and incubated at a temperature of 40 C.

Subcultures into fresh media, prepared as previously described, were made each week on Monday, Wednesday, and Friday. Just before making the transfers, the pH of each parent culture was determined by the use of pH test papers. If the pH was less than 6, from 0.05 ml to 0.1 ml of a sterile 5 percent solution of $NaHCO_3$ was added to the subculture. The growth of this strain of *Histomonas* occurred in a pH range of about 5.8 to 6.8. The large quantities of antibiotics that were used kept most of the cultures free of multiplying bacteria.

A number of substitutes for blood serum were tested. Of these the only one which gave results approximating that of serum was fresh cream, either pasteurized or non-pasteurized, sterilized by passage through a Selas filter (maximum porosity radius 0.6 micron). The filtered cream was used in quantities of 0.5 ml per culture. In 75 cultures the histomonad growth ranged from 1 per 20 microscopic fields (15× by 20×) to 32 per field; the average was 1.6 protozoa per field. In 128 cultures containing serum the range was from 1 per 13 fields to 34 per field; the average was 1.8 protozoa per field. Cream is now being used in place of serum for routine culture purposes.

Fresh cow's milk similarly sterilized was tried in place of cream, but although histomonad growth occurred it was not satisfactory. It was also observed that the histomonads could not be maintained in culture if reconstituted "non-fat" powdered milk was substituted for serum. This served to indicate that one of the growth factors may be lipid in nature. Histomonad growth also could not be maintained when protein-free bovine serum ultrafiltrate was used in place of serum; this in turn may indicate the protein nature of another growth factor.

The present studies also show that histomonads will grow in the basal medium described, although not well, if the following is added in lieu of the serum: 0.3 ml of a sterile 10 mg per ml aqueous suspension of palmitic acid and/or 0.3 ml of a sterile 2.5 mg per ml aqueous suspension of cholesterol (or dihydrocholesterol).—ELLIOTT LESSER, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

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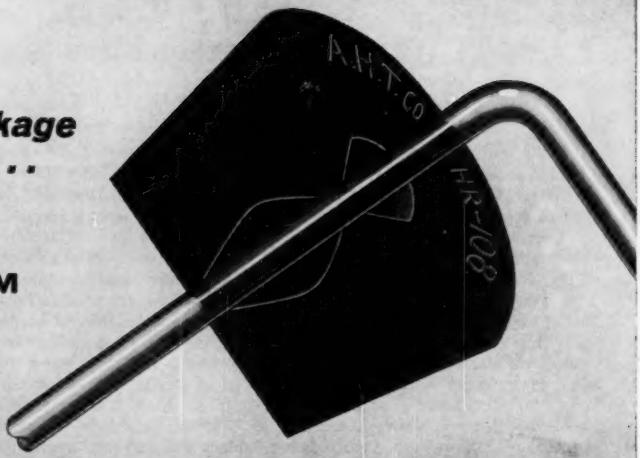

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4	44	48	1.81	6.60
5	37	36	2.02	5.52
5½	31	36	2.18	5.94
6	27	30	2.33	5.29
6½	24	30	2.49	5.68
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8	16	18	3.12	4.26
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2	74	72	1.58	8.65
3	55	60	1.79	8.15
4	46	48	1.87	6.79
5	38	36	2.04	5.58
5½	33	36	2.28	6.23
6	28	30	2.42	5.52
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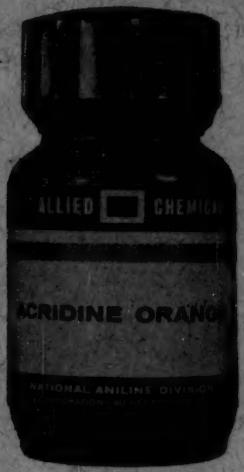

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